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PROCEEDINGS OF THE BIOCHEMICAL SOCIETY

The **285th Meeting** *of the Society was held in the Department of Biochemistry and Pharmacology, University College, Dublin, on Friday,* 14 *April* 1950, *when the following papers were read:*

COMMUNICATIONS

Oxalacetic Decarboxylase. By D. HERBERT. (*National Institute for Medical Research, Mill Hill, London*)

Krampitz & Werkman (1941) discovered that the organism *Micrococcus lysodeikticus* contains an enzyme, subsequently named 'oxalacetic decarboxylase', that catalyses the decomposition of oxalacetic acid to pyruvic acid and CO_2. There have been suggestions that this enzyme is concerned in the fixation of CO_2 by carboxylation of pyruvate, and that biotin is implicated in the process.

Highly purified preparations of this enzyme have been obtained. The enzyme was liberated from the cells of *M. lysodeikticus* suspensions by treatment with crystalline lysozyme, a procedure previously used in the isolation of crystalline catalase from this organism (Herbert & Pinsent, 1948). It was purified by fractionation with acetone at $-5°$, precipitation at pH 3·8, and repeated fractionation with $(NH_4)_2SO_4$. The purest preparations obtained had a Q_{CO_2} of 1,200,000, compared with $Q_{CO_2} \sim 200$ for the starting material. The overall yield of enzyme was *c.* 30 %. (The activity of the enzyme was determined manometrically, by measuring the CO_2 evolved from oxalacetate (M/100) in the presence of enzyme and $MnSO_4$ (M/1000) at pH 5·6 and 30°.)

Such preparations are more active than the majority of pure enzymes, and crystallize from $(NH_4)_2SO_4$ as fine needles. Nevertheless, they are not homogeneous in the ultracentrifuge, which reveals three distinct components. It is estimated that the pure enzyme must have a Q_{CO_2} of at least 3×10^6, and can account for no more than 0·006 % of the weight of the bacterium. This is in contrast to the catalase content of the same organism, which can attain 2 % of the total dry weight.

The purified enzyme is completely inactive towards oxalacetate in the absence of metallic cations; it is activated by a number of divalent cations (Mg^{++}, Cd^{++}, Co^{++}, Ni^{++}, etc.), of which Mn^{++} is the most effective. Trivalent cations (La^{+++}, Al^{+++}, Fe^{+++}, Cr^{+++}) give negligible activation. These results are similar to those previously found for the pyruvic decarboxylase of yeast (Green, Herbert & Subrahmanyan, 1941).

The purified enzyme is not activated by biotin, and so far all attempts to demonstrate the presence of a prosthetic group have given negative results.

A mixture of oxalacetic decarboxylase, malic dehydrogenase, Co I, and Mn^{++}, with ferricyanide as H-acceptor, brings about oxidative decarboxylation of malate to pyruvate and CO_2. It is suggested that CO_2-fixation may be due to the reversal of this reaction sequence.

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The Reaction of Vitamin D and other substances with Iodine Trichloride. By J. GREEN. (*The Research Laboratories, Vitamins Ltd.,* 23 *Upper Mall, London, W.* 6)

During investigations into the reactions of some sterols with inorganic halides, it was discovered that iodine trichloride reacted with the vitamins D in cold carbon tetrachloride solution to form a deep pink colour, which was shown, on spectroscopic evidence, to be due to the liberation of free iodine. Excess reagent reabsorbed the iodine with the formation of iodine monochloride. The reaction takes place instantaneously and is given by ergosterol, 7-dehydrocholesterol, caliciferol, vitamin D_3, lumisterol, tachysterol, the suprasterols, vitamin A and β-carotene. The reaction is not given by cholesterol or the sitosterols.

Outside the series of vitamin D irradiation sterols, the only substances found to react in this remarkable way are those containing systems of three conjugated double bonds. Other systems either do not react or absorb the whole iodine trichloride molecule.

Spectroscopic measurements at 518 mμ. showed that on adding increasing amounts of ICl_3 to a constant amount of vitamin D_3, the amounts of iodine liberated increased to a peak value and then slowly decreased due to reabsorption of the iodine. Determination of the peak value showed that, in all cases where the reaction was positive it was truly stoichiometric, and the reagent quantitatively chlorinated an integral number of double bonds, liberating stoichiometric quantities of iodine. The back reaction of recombination with excess reagent to form iodine monochloride is not stoichiometric, several moles excess ICl_3 being necessary to remove all the iodine, determination of the end-point of the reaction by measurement of the maximum iodine colorimetrically is thus fairly easy.

Titration is best carried out on the Spekker microabsorptiometer, using Spectrum Green 604 filters, adding increasing amounts of ICl_3 to a constant amount of vitamin D, until the peak reading is reached. If the peak readings are plotted against micrograms of vitamin D, a straight-line curve is obtained. Using 0·5 ml. cells, quantities from 20 to 50 μg. can be titrated.

Vitamin D_3 titrates to three double bonds, as does, preferentially, calciferol; one sample of calciferol apparently reacted with all four double bonds. Ergosterol always titrates to three bonds, but its isomer, lumisterol, only two. The suprasterols react with two bonds, in spite of their reputed non-conjugated structure, and tachysterol reacts with rather less than two bonds. Vitamin A and β-carotene react with five and eleven bonds respectively.

Study of the reaction mechanism indicates that, in all cases, a two-stage reaction is involved.

Gastric Urease and HCl Secretion. By R. E. Davies and H. L. Kornberg. (*Medical Research Council Unit for Research in Cell Metabolism, Department of Biochemistry, The University, Sheffield* 10)

Mechanisms have been suggested by which gastric urease hydrolyses blood urea to provide NH_4^+ ions which act as precursors of H^+ ions either by the reaction $NH_4^+ \rightarrow NH_3 + H^+$ (Rigoni, 1930; cf. Mathews, 1925), or by an exchange of NH_4^+ and H^+ ions between mucosae and blood during acid secretion (Glick, 1949).

These hypotheses can be tested by experiments *in vitro*, when the amount of available urea is known. Isolated frog gastric mucosa (Davies, 1948*a*) and mouse stomach (Davies, 1948*b*; Davenport & Jensen, 1948, 1949) can secrete 20–40 μl. HCl/mg. dry wt. in the absence of added urea. However, the tissue store of urea might be large enough to account for this acid, and experiments were carried out to investigate this possibility.

Frog gastric mucosae and whole mouse stomachs were washed, dropped into boiling water and boiled for 4 min. before manometric estimation of the urea content with jack-bean urease. Frog mucosa (ten samples) contained 0·8–0·9 μl./mg. dry wt.; average 0·88 μl. (40–45 mg. % of wet wt.); whilst whole mouse stomach (five samples) contained 0·3–0·4 μl./mg. dry wt., average 0·34 μl. (15–20 mg. % of wet wt.).

The urease activity of freshly ground samples of both tissues was measured manometrically as Q_{urea} (μl. urea hydrolysed/mg. of tissue dry wt./hr.). Ground frog mucosa (ten samples) had Q_{urea} values of 0–1·5, average 1·1; ground mouse stomach (ten samples) had values of 3·6–6·8, average 5·2. (Experiments at 38° with $2{\cdot}5 \times 10^{-2}$M-urea in 0·2M-acetate buffer, pH 5·0.)

FitzGerald & Murphy (1948) found that urea decreased the gastric response to histamine in man, and Davenport & Jensen (1949) found that urea did not increase the amounts of acid secreted by isolated mouse stomachs, but that about six times more acid than ammonia was formed.

The HCl produced by 32 isolated frog gastric mucosae was determined, when urea was added initially, or during the experiments, to concentrations of 2, 10, or 20 mM/l.; no detectable stimulation or inhibition of the rate of acid secretion or respiration occurred. The amounts of ammonia produced during 3 hr., estimated according to Conway (1947), were not detectable, i.e. < 2 μl. (12 mucosae). The urea added was also determined in four cases and was recovered quantitatively after the incubation.

Whilst Q_{HCl} values of 3·6 at 25° were obtained with these (winter) frog stomachs, the Q_{urea} values were $< 0{\cdot}03$, so the added urea was not utilized for acid production. Though some urease activity was found in ground frog gastric mucosa, there was none in these 32 experiments on intact tied bags of tissue. Since there is no evidence that urea is synthesized by gastric mucosa (Krebs, 1934; FitzGerald & Murphy, 1948), and the urea content of the tissue is more than 20 times too small to account for the acid produced, it follows that gastric urease plays no part in the secretion of acid by the isolated frog or mouse stomach (cf. Davenport & Jensen, 1949). This view is now accepted by Glick (1950). Any acid-neutralizing role of the urease during these experiments is also excluded (cf. FitzGerald, 1946).

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The Azide effect in Yeast with respect to Potassium and Phosphate Permeability. By E. J. Conway and P. T. Moore. (*Department of Biochemistry and Pharmacology, University College, Dublin*)

In resting bakers' yeast, external K ions labelled with ^{42}K mix with the yeast K at a very slow rate (in one experiment about 0·25 mmol. K ions entered 1 kg. yeast in 39 min. from N/50-KCl). In fermenting yeast (1:1 of 5% glucose containing KCl) K enters very rapidly as shown by the ^{42}K counts, and in agreement with the chemical analyses (Conway & O'Malley, 1944, 1946). From N/50-KCl about 12 mmol. K ions enter in 30 min.

Azide (M/200, at pH of 5–6) produces a considerable inhibition of the K entrance in accordance with the chemical analyses, but does not completely inhibit it. The effect of azide added at 20 or 30 min. after the onset of fermentation is to produce a rapid exit of K from the cells so that the external fluid count increases. The K ions returning to the external fluid have a specific count which indicates that the absorbed ions mix, almost, if not quite fully with the intracellular potassium.

Besides this effect of azide on K, the increased acidity produced by the fermentation falls to near zero due to the entrance of H ions into the cells.

If yeast is mixed with unbuffered 5% glucose (1:1) without any KCl, and azide introduced after 20 or 30 min., the titratable acidity produced by the excretion of free succinic acid (amounting to about 40 m-equiv./l. of external fluid) almost disappears, but no succinic acid returns to the cells, but remains outside as potassium succinate, K ions having exchanged for H ions.

The maximum PO_4 entrance into yeast fermenting under such conditions (with $KHPO_4$ included) was found to be only about 6% of the maximum K entrance. As shown with labelled phosphate the absorption is inhibited by azide confirming the observations of other workers (e.g. Spiegelman, Kamen & Sussman, 1948). Also the azide effect on the K and H exchange, on the excretion of succinic acid, or the acid-alkali mechanism (see Conway, 1949) is quite independent of the presence of external phosphate. The evidence suggests a direct effect on the special catalyst concerned rather than an indirect one through the inhibition of special phosphorylations (Spiegelman *et al.* 1948).

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Concerning the Entrance Rate of KCl into the whole Isolated Sartorius of the Frog and into Single Fibres. By E. J. Conway, Mary Carey and P. T. Moore. (*Department of Biochemistry and Pharmacology, University College, Dublin*)

It was previously found that the entrance rate of KCl into the isolated sartorius could be readily followed by periodic observation of the volume change when the muscle was immersed in isotonic Ringer solutions with KCl in relatively large part replacing NaCl (Conway & Moore, 1945; Conway, 1947). Expressing such entrance rate by the half-period of the complete process (which is convenient for comparative purposes) it was found to be independent of the concentration of KCl (which implies that the KCl is accumulated at a rate proportional to its concentration). Replacing of the Cl ions by larger anions to which the membrane is practically impermeable, the rate of KCl entrance was found to be proportional to the product of K- and Cl-ion concentrations.

The half-period of entrance at room temperature was found to be approximately 40 min., and at 0° 120 min.

It was surprising to find with the single fibres that the half-period at 0° was as low as 12 min. This rate was not appreciably affected by using immersion fluids containing 50 % of fresh frog serum.

Though occasional fibres were observed which showed no volume change (when a very appreciable amount could be expected), the general volume change was of the expected order. The single fibre slowly contracts in length on immersion in the high KCl solutions, though as a rule not so much as to interfere seriously with an estimate of the half-period. Such contraction could be perfectly controlled by curarizing the frog beforehand. Under such conditions the fibres remained exactly the same length for relatively long periods, although a considerable increase in cross-section—often by as much as 60 %—could occur.

It was found that on sectioning the fibres transversely to lengths as short as 0·6 mm. they behaved like fibres of 10 mm. length, or more, swelling transversely to the same degree but not changing in length (curarized frog).

The entrance rate of ^{42}K into single fibres is being investigated. It has already been examined for the whole sartorius by Harris & Burn (1949).

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Microdiffusion Methods. Determination of Acetic Acid in Biological Fluids. By E. J. Conway and Mary Downey. (*Department of Biochemistry and Pharmacology, University College, Dublin*)

Acetic acid and the lower aliphatic monocarboxylic acids may be readily determined by a microdiffusion procedure (Conway, 1947).

Owing, however, to the very low tensions they exhibit, the diffusion process is very slow, even at raised temperatures, unless specially treated to increase the tension. After trying the effect of a large number of substances it was found that the greatest increase of tension was produced by finely powdered anhydrous sodium sulphate. With this the full diffusion period may be reduced from some days to 4–5 hr. at room temperature.

In the procedure 0·1 ml. of the fluid is pipetted into the central chamber of a Conway unit (no. 1) on to the surface of a suitably cut circular piece of thin filter-paper resting on the floor of the chamber (this is done in order to spread the fluid fully and easily); 0·1 ml. of N-sulphuric acid are then pipetted in a similar way on to the surface of the central chamber. If the fluid for examination contains CO_2, 5–10 min. exposure may now be allowed (no appreciable acetic acid escapes in this time).

Into the outer chamber 2 ml. of a suitably weak sodium citrate solution is pipetted to absorb the acetic acid (pure distilled water may even be used). Then the inner chamber of the unit is quickly covered with powdered anhydrous sodium sulphate using a small spoon spatula. A thick layer is so formed, spread out and pressed down quickly by the spatula. The lid with fixative is now placed in position, and after 5 hr. at room temperature, the contents of the outer chamber are either directly titrated (using phenolphthalein) or after removal (with a little washing) to a small tube.

The method is very delicate and accurate, and may be applied to normal blood, using 0·5 ml. (the filter-paper technique being then not used). Using 0·5 ml. 8 hr. at room temperature are allowed (or overnight for convenience) for the absorption.

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Conditions determining the excretion of pyruvic acid by fermenting yeast. By E. J. CONWAY and F. C. WINDER. (*Department of Biochemistry and Pharmacology, University College, Dublin*)

It has been shown that when bakers' yeast ferments unbuffered 5% glucose (1:1) there is an excretion of organic acid which within 30 min. is of the order of 40 m.equiv./l., of the external fluid, and is almost altogether succinic acid, with only a trace of pyruvic and possibly traces of other acids (Conway & O'Malley, 1946). When the solution is maintained at a pH of 5·5–6·0 by the addition of N/1-sodium bicarbonate throughout the fermentation, the succinic acid excretion is increased by about 15 m.equiv./l., and the pyruvic acid is increased from a trace amount up to about 6 mmol./l. The extra acidity arising from the raised pH is largely accounted for by the increased succinic and pyruvic acids.

On the other hand, when the mixture is buffered with M/5-sodium citrate, the increase in non-volatile acid excreted at a pH of 6 over that at a pH of 3 is considerable (about 50 m.equiv./l. or more). Less than half of this increase is due to succinic and pyruvic acids though the latter appears somewhat increased over that associated with the use of bicarbonate.

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A Simplified Method for the Isolation of Thymidine from Thymonucleic Acid. By T. G. BRADY. (*Department of Biochemistry and Pharmacology, University College, Dublin*)

The method depends on the solubility of thymidine in alcohol and acetone and avoids the time-consuming removal of inorganic ions employed in earlier isolations (Klein, 1938; Brady, 1941).

(1) Aqueous extracts of acetone-dried calves' mucosa are used instead of glycerine extracts.

(2) After dephosphorylation the solution is clarified by centrifuging and is concentrated to dryness by vacuum distillation.

(3) The powdered residue is extracted by shaking at room temperature with four times its weight of 95% alcohol. The extraction is repeated until only a faint diphenylamine colour test is obtained. The combined extracts are dried by vacuum distillation.

(4) The yellowish powder so obtained is re-extracted about ten times with acetone at room temperature. On concentrating the combined extracts, impure thymidine immediately crystallizes out; m.p. 170–80°.

(5) One recrystallization from 50% aqueous alcohol gives pure thymidine; m.p. 185–6°.

In one experiment in which there was only 50% dephosphorylation, 330 mg. thymidine were obtained from 10 g. thymonucleic acid.

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A Study of the Low Molecular Constituents of the Yeast Cell. By T. G. BRADY. (*Department of Biochemistry and Pharmacology, University College, Dublin*)

Protein-free extracts of yeast were prepared by boiling the suspension for a short time and centrifuging. The clear fluid so obtained was used to study (1) the electrometric titration between pH 2 and 12, (2) amino-acids, (3) total soluble phosphate, (4) inorganic, acid-labile and soluble organic phosphate, (5) keto-bodies, (6) citric acid, (7) succinic acid, (8) alcohol.

The determinations were made on (*a*) resting yeast suspension; (*b*) yeast suspension fermenting 5% glucose for 20 min.; (*c*) yeast suspension after fermentation and standing for 20 hr. The determinations were repeated on yeast from the same batch after oxygenation for 48 hr.

The electrometric titration shows that there is strong buffering below pH 5, and between pH 8 and 11. From pH 6 to·8 there is minimal buffering. Below pH 5 the buffering is due to phosphorylated compounds and organic acids such as succinic and citric, but the surprisingly large amounts of free

amino-acids which are clearly indicated by the heavy buffering in the region above 8 also contribute considerably to the buffering in this region.

Amino-acids. Free amino-acids in a concentration of approximately 140 m-equiv./kg. of fresh yeast (i.e. about 16 g./kg.) were demonstrated by Folin's method. Chromatography on paper gave five spots, four of which have been identified as glutamic acid, aspartic acid, glycine and alanine, the fifth is probably leucine. There is no quantitative alteration in the total amino-acid concentration during fermentation. Oxygenation, however, leads to a definite increase.

Phosphate. Oxygenation leads to a slight increase in total soluble phosphate, and approximately 60% increase in organic phosphate.

Keto-bodies. The average ratio of 1·8 for absorption at 420 and 520 mμ. (Friedemann & Haugen, 1943) indicates that glyceraldehyde or a similar substance is present in resting yeast. In actively fermenting yeast the ratio changes to 1·2, showing that pyruvic acid is formed. Fermentation causes a five-fold increase from the resting level of 0·5 to 2·6 mmol.

Citric acid was present in all the yeast samples examined, and remained remarkably constant. Even after oxygenation its concentration was the same as that in fresh yeast.

Succinic acid. As previously shown (Brady, 1948), succinic acid can be removed from yeast by oxygenation. In both fresh and oxygenated yeast the increase in the suspension after fermentation and standing for 20 hr. is practically identical, showing that the precursor is not removed by oxygenation.

Yeast fermented with 3, 5 and 10% glucose gave less succinic acid with the high concentration of glucose than with the lower concentrations, while alcohol increased in a direct relation with the sugar concentration. This is considered as evidence that the immediate precursor of succinic acid in bakers' yeast is not glucose.

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Vitamin A in the Kidney of the Rat. By Eva Eden and T. Moore. (*Dunn Nutritional Laboratory, University of Cambridge and Medical Research Council*)

Johnson & Baumann (1947*a*, *b*) reported that when rats were kept on a diet deficient in vitamin A, with supplements of 35 i.u. of carotene daily, or of small doses of preformed vitamin A, more vitamin was accumulated in their kidneys than in their livers. With higher doses, however, the usual predominance of the liver as the main site of storage was asserted.

In the present experiments the ability of the kidneys to absorb greater concentrations of vitamin A than the liver was confirmed in young rats given low doses of vitamin A acetate in conjunction with a basal diet deficient in vitamin A. Thus (Table 1) it will be seen that in Exp. 1, with daily doses of 20 i.u., the mean concentration of vitamin A in the kidneys, as estimated by the $SbCl_3$ method, was more than five times its level in the liver. Greater concentrations of vitamin A were found in the kidneys than in the livers in all the animals examined, while in seven out of nine instances the total amount of vitamin in the kidneys exceeded the amount present in the liver.

When the level of dosing was raised by giving a single large dose of vitamin A acetate to rats previously deprived of the vitamin (Exp. 2) by giving large daily doses of cod-liver oil for 4 or 7 weeks (Exps. 3 and 4) or by giving a liberal diet of natural foods for about 1 year (Exp. 5) the stores of vitamin A found in the liver invariably exceeded those found in the kidneys. Under the experimental conditions chosen, moreover, the larger intakes of vitamin A had no effect in raising the concentration of vitamin in the kidneys. Quite unexpectedly the kidneys

Table 1

Exp.	No. of rats	Daily dose of vitamin A (i.u.)	Form of vitamin A	Period of dosing	Vitamin A (i.u. total) Liver Range	Liver Mean	Kidney Range	Kidney Mean	Vitamin A (i.u./g.) Liver Range	Liver Mean	Kidney Range	Kidney Mean
1	9	20	Vit. A acet.	4–5 weeks	26–330	90·6	59–200	97·7	2·3–42	11·3	35–140	64
2	6	2,000	Vit. A acet.	1 day	240–550	335	10·2–41	23·7	34–69	49·3	11–23	15·2
3	4	2,000	c.L.o.	4 weeks	3,860–7,490	6,090	6·1–21·8	14·3	610–1,240	870	6–13·5	9·9
4	4	2,000	c.L.o.	7 weeks	7,850–57,000	26,400	1·7–57	32·2	1,240–6,700	3,340	1–33	15·9
5	8	—	Nat. diet	*c.* 1 year	1,600–4,560	3,140	15–52	32·8	110–400	278	8·5–27	17·1

Vit. A acet. = vitamin A acetate. c.L.o. = cod-liver oil. Nat. diet = mixed diet of natural foods.

contained most vitamin A in Exp. 1, in which the lowest amounts of vitamin A were administered. In Exps. 2, 3, 4 and 5 the mean concentrations of vitamin A in the kidneys only varied between 9·9 and 17·1 i.u./g., as compared with 49–3340 i.u. for the mean concentrations in the liver. Previous work has shown, however, that with doses of vitamin A approaching the toxic level much higher concentrations in the kidneys are readily attained (Davies & Moore, 1934).

Preliminary experiments on the influence of sex have suggested that the amounts of vitamin A in the kidneys tend to be greater in male than in female rats.

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The Quantitative Analysis of Deoxypentose Nucleic Acids. By G. R. Wyatt (introduced by R. Markham). (*Agricultural Research Council Plant Virus Research Unit, Molteno Institute, Cambridge*)

A method simpler than that of Vischer & Chargaff (1948) has been developed for the quantitative analysis by paper chromatography of the purine and pyrimidine bases in deoxypentose nucleic acids. The bases freed by hydrolysis in formic acid at 175° for 30 min. are separated on one-dimensional chromatograms, using as the solvent an aqueous solution containing *iso*-propanol 65% (v/v) and 2·0N-HCl. The spots are detected by the photographic method of Markham & Smith (1949), eluted, and estimated spectrophotometrically.

In addition to adenine, guanine, thymine, and cytosine, certain nucleic acids contain 5-methyl-cytosine, which has been isolated and identified by deamination to thymine and by comparison with synthetic 5-methyl-cytosine. It is present in all the animal deoxyribonucleic acids yet analysed (from mammals, fish, and insects) in small amounts characteristic of the source, and in nucleic acid from wheat germ, but it has not been detected in microbial nucleic acids. In agreement with Vischer, Zamenhof & Chargaff (1949), and contrary to Johnson & Coghill (1925), none could be found in nucleic acid from tubercle bacilli.

The following are preliminary results:

Molar proportions of the bases in deoxypentose nucleic acids*

Source	Adenine	Guanine	Thymine	Cytosine	5-Methyl-cytosine
Calf thymus	1·10	0·86	1·15	0·89	0·052
Beef spleen	1·12±0·006†	0·86±0·006	1·16±0·008	0·86±0·011	0·052±0·002
Ram sperm	1·14	0·89	1·11	0·86	0·042
Herring sperm	1·10±0·015	0·91±0·009	1·16±0·021	0·86±0·006	0·073±0·002
Locusts (whole)	1·15	0·82	1·18	0·86	<0·01
Wheat germ ('Bemax')	1·10	1·00	1·16	0·74	0·23
Tubercle bacilli (human and bovine)	0·70	1·16	0·78	1·36	0·00
Escherichia coli	0·92	0·81	1·20	1·06	0·00
E. coli bacteriophage T_5	1·30	0·83	1·45	0·42	0·00
Virus of gypsy moth polyhedral disease	0·86	1·20	0·92	1·02	0·00

* Calculated to an arbitrary total of 4·00 (excluding methyl-cytosine).
† Standard error of the mean of six or seven independent sets of estimations.

The similarity in composition of the animal deoxyribonucleic acids is very striking, although the differences between those from different species are statistically significant. Microbial nucleic acids show much greater divergences. The possibility must be considered that the preparations may not be homogeneous, but attempts at fractionation have not yet succeeded.

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The Metabolism of ^{15}N-labelled Urea in the Cat. By R. E. DAVIES and H. L. KORNBERG. (*Medical Research Council Unit for Research in Cell Metabolism, Department of Biochemistry, The University, Sheffield* 10)

Until recently urea has been regarded as solely an end-product of mammalian nitrogen metabolism. Bloch (1946) tested this point by feeding ^{15}N-labelled urea to rats. He obtained a slight incorporation of ^{15}N in the proteins of the liver and the intestine but regarded this as the result of bacterial decomposition of urea in the gut. On the other hand, Leifer, Roth & Hempelmann (1948), who injected ^{14}C labelled urea into mice, recovered 20·8 % of the injected ^{14}C in the exhaled CO_2 and were thus led to assume that urea is not a stable end-product in the mammalian body. This has been confirmed by Skipper (1949).

Table 1 gives some of the results. The isotope content of all other tissues was between 0·003 and 0·008 atom % excess ^{15}N and accounted for 10 % of the injected ^{15}N. The amide-N fraction of the liver was significantly enriched and 2·3 % of the injected ^{15}N appeared in the urine in a form other than urea or ammonia.

These results show that at least 2·5 % of the urea N was metabolized. This figure is a minimum; Berenbom & White (1950) have shown that if ammonium glutamate, ^{15}N-labelled in the ammonium group, is injected intravenously into rats, most of the isotope is rapidly excreted as urea and that there is only slight incorporation into liver and kidney proteins. Furthermore, since any ammonia arising from the hydrolysis of urea will be largely reconverted to urea in the liver, the amount of isotope found in the non-urea non-ammonia fraction of the urine can represent only a minimum figure for the metabolism. The present results are therefore not incompatible with the value of 20·8 % breakdown obtained by Leifer *et al.* (1948), which also represents a minimum. The low isotope content of gastric mucosa suggests that the urea-urease system of this tissue plays no part in local synthetic mechanisms.

Table 1. *Distribution of* ^{15}N *after injection of* 215 *mg.* ^{15}N*-labelled urea* (32 *atom* % *excess*) *into a cat*

Fraction	N content (g./100 g.)	Total N (g.)	^{15}N content (atom % excess)	Percentage of injected ^{15}N	Method of estimation
Urine total N	0·94	2·35	1·075	81	Kjeldahl N
Urine urea N*	0·73	1·83	1·287	75	(*a*) Fosse (1916), Kiech & Luck (1928); (*b*) Van Slyke & Cullen (1916)
Urine ammonia N	0·05	0·13	0·042	0·17	Van Slyke & Cullen (1916)
Urine non-urea non-ammonia N	0·21	0·53	0·136	2·3	Kjeldahl N on urea- and ammonia-free urine
Liver total N	2·84	1·66	0·008	0·42	Kjeldahl N
Liver amide N	0·12	0·08	0·013	0·04	Schoenheimer, Ratner & Rittenberg (1939)
Gastric mucosa total N	2·04	0·15	0·003	0·01	Kjeldahl N

Recovery of injected ^{15}N: 92 %.

* Estimated by two different methods. Accuracy of mass spectrometer: ±0·003.

The following experiments were undertaken with reference to the problem of the role of gastric urease. ^{15}N-labelled urea, synthesized according to Cavalieri, Blair & Brown (1948), was injected subcutaneously into a cat weighing 2·0 kg., kept on a low-protein diet. The cat was chosen because the urease activity of ground gastric mucosa is much higher in cats than in rats (Luck, 1924; FitzGerald, 1946). The dose was 98·8 mg. urea on the first day and 116·4 mg. on the second. The urine was collected in conc. HCl under toluene. On the third day the cat was killed; the tissues were rapidly removed and stored at −12°. The ^{15}N content of various fractions of urine, blood, brain, diaphragm, heart, intestinal tract, kidneys, liver, skin and hair, spleen and stomach was measured by mass spectrometry.

We are greatly indebted to Dr R. Bentley, who carried out the mass-spectrometric analyses.

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The Metabolism of Urea in Animal Tissues. By O. FITZGERALD (introduced by E. J. CONWAY)

Of recent years there has been a renewal of interest in the metabolism of urea in the tissues. The work of Bloch (1946) suggested that urea, tagged with ^{15}N, was not metabolized in the tissues and that administered urea, so tagged, appeared quantitatively (at least 95% of it) in the urine. However, more recently Liefer *et al.* used ^{14}C tagged urea and estimated the amount of ^{14}C as CO_2 in the expired air. They found that urea was metabolized to the extent of about 20%. It should be noted that the first group of workers used rats as the experimental animals; Leifer, Roth & Hempelmann (1948) used mice. Urease is definitely present in the stomach (Luck, 1924; Luck & Seth, 1924, 1925), and we have recently studied its general distribution (FitzGerald, 1946; FitzGerald & Murphy, 1950). The stomach has the largest concentration of urease and the amount in the gastric mucosa may be quite large in some species. There is a definite species difference. Urease, however, appears to be present in other organs such as the kidney and the liver. The ferment activity of these tissues is less than that of the stomach. We have attempted to follow its comparative distribution in different species (see Table 1). The certification of urease activity in an organ like the liver presents certain problems which we have attempted to solve but we are not completely satisfied with the results.

Table 1. *Urease activity of liver, kidney and stomach expressed as micrograms ammonia-N released per g. of wet tissue per hour at* 37°

Animal	Liver	Kidney	Stomach
Dog (1)	136 (=at least 4·5 mg./hr.)	—	3,160 (=at least 30 mg./hr.)
Cat (2)	376	355	1,210
Mouse (11)	35	69	2,860
Rat (3)	27	12	296
Frog (6)	17	34	201

The presence of urease in tissues seems to support the view that urea may enter into intermediary metabolism. Gastric urease, we think, is of importance in the protection of the mucosa and the neutralization of hydrochloric acid. In general, urease acting upon urea will produce ammonia which may be available for transamination.

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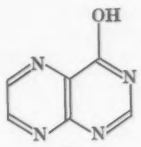
The Avidity of Folic Acid and other Pteridines for the Ions of Heavy Metals. By A. ALBERT. (*The Australian National University, 183 Euston Road, London, N.W. 1*)

It has recently been pointed out (Albert & Brown, 1949) that all the naturally occurring pteridines contain a hydroxyl group in the 4-position, and hence have a configuration which should make them combine tenaciously with the ions of heavy metals. In order to obtain a quantitative measure of this avidity, stability constants for the following type of equilibrium were determined potentiometrically in aqueous solutions at 20°.

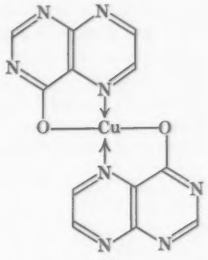
$$K_s = \frac{[\text{complex}]}{[\text{metal ion}]\,[\text{free anion}]^2}.$$

First of all, stability constants were obtained for 4-hydroxypteridine (I) because it possesses the metal-binding configuration in its simplest form. It gives complexes of the type (II). The constants for this substance and for folic acid are given in Table 1. It is assumed that folic acid binds metals in the same way as the parent substance (I).

The copper complexes of both pteridines are green, the cobalt are deep yellow and the iron are intense blood red. Xanthopterin behaves similarly.



(I)



(II)

Table 1 shows that the stability constants of the pteridines are, in some cases, less than those of typical amino-acids. This is particularly so in the

Table 1. *Log stability constants*

	Cu^{++}	Ni^{++}	Zn^{++}	Co^{++}	Cd^{++}	Fe^{++}	Mn^{++}	Mg^{++}
4-Hydroxypteridine	9·5	7·8	*c.* 5·9	6·7	*c.* 5·9	5·9	4·5	<4
Folic acid	7·8	—	—	8·1	—	7·9	—	—
Proline	16·8	11·3	10·2	9·3	8·7	8·3	*c.* 5·5	<4
Glycine	15·4	11·0	9·3	8·9	8·1	7·8	5·5	*c.* 4
Asparagine	14·9	10·6	8·7	8·4	6·8	6·5	*c.* 4·5	*c.* 4

Table 2. *Number of molecules of chelating agent (in* 0·01 M *solution) bound by one atom of metal* (0·005 M)

	Cu^{++}	Ni^{++}	Zn^{++}	Co^{++}	Cd^{++}	Fe^{++}	Mn^{++}	Mg^{++}
4-Hydroxypteridine (pK = 7·9)	2·0	1·8	*c.* 1·0	1·2	*c.* 1·0	1·0	0·2	<0·02
Proline (pK = 10·7)	1·9	1·0	0·5	0·4	0·1	0·1	0·02	<0·02
Glycine (pK = 9·9)	1·9	1·4	0·9	0·7	0·4	0·3	0·04	0·02
Asparagine (pK = 8·9)	2·0	1·7	1·3	1·2	0·7	0·5	0·14	0·1

case of copper. In spite of this, the pteridines can, because of their high degree of ionization at pH 7·3, successfully compete with these amino-acids, as Table 2 shows. The low pK of 4-hydroxypteridine (7·9), furnishes it with a larger proportion of chelating ions at pH 7·3 than is possible for the amino-acids. This produces a greater quantity of metal-complex, according to the mass action law. Hence, as may be seen from Table 2, 4-hydroxypteridine competes for copper and cobalt on equal terms with asparagine (the most successful of those amino-acids that have only two ionizing groups; Albert, 1950). More important still, it can out-compete any of the amino-acids for iron and manganese.

The high stability constants of folic acid in respect of cobalt and iron, coupled with its high degree of ionization (pK = 8·3) combine to make it a powerful competitor for these two metals. The equal affinity of folic acid for copper and for iron is quite unprecedented among complex-forming substances.

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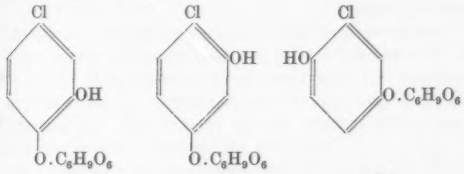
PROCEEDINGS OF THE BIOCHEMICAL SOCIETY

The **286th Meeting** *of the Biochemical Society was held in the Physiology Institute, Newport Road, Cardiff, on Friday,* 19 *May* 1950, *when the following papers were read:*

COMMUNICATIONS

The Orientation of Glucuronic Acid Conjugation in Resacetophenone. By K. S. Dodgson. (*Physiology Institute, Newport Road, Cardiff*)

Dodgson & Williams (1949) and Dodgson, Smith & Williams (1950) have shown that the dihydroxybenzene derivatives, 4-chlorocatechol, 4-chlororesorcinol and chloroquinol, form, in rabbits, monoglucuronides which may be formulated as (I), (II) and (III) respectively.



(I) (II) (III)

In all these compounds, conjugation with glucuronic acid occurs on the hydroxyl group which is farthest away from the chlorine atom.

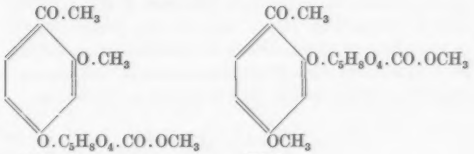
It has now been shown that resacetophenone (IV) behaves in similar fashion.

Resacetophenone glucuronide has been isolated previously from the urine of dogs and rabbits by Nencki (1894), who described it as a monohydrated monoglucuronide which melted at approximately 170°.

In the present experiment, this glucuronide was isolated in excellent yield (corresponding to 35 % of the dose) from the urine of rabbits receiving resacetophenone orally. The glucuronide was a monohydrate, m.p. 175–176° (with decomposition) and $[\alpha]_D^{22°} - 99°$ (0·714 % in water). It gave a deep red-purple colour with $FeCl_3$, and an intense blue colour with 2:6-dichloroquinone chloroimide in the presence of Na_2CO_3.

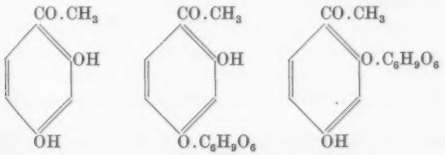
The glucuronide could be one of two compounds, 4-(β-D-glucuronosido)-2-hydroxyacetophenone (V) or 2-(β-D-glucuronosido)-4-hydroxyacetophenone (VI), depending on which hydroxyl group had been conjugated.

The procedure used to establish which of these two possibilities was the correct one was similar to that described in earlier papers in this series (Dodgson & Williams, 1949), and consisted of partial methylation of the glucuronide to give either (VII) or (VIII). Subsequent hydrolysis of VII would give 4-hydroxy-2-methoxyacetophenone (IX), whereas hydrolysis of VIII would give 2-hydroxy-4-methoxyacetophenone (X). Both these compounds are readily characterizable.

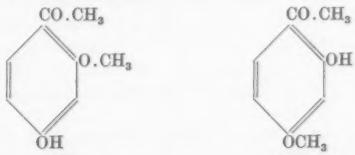


(VII) (VIII)

The pure glucuronide was methylated (as a suspension in ethanol) with ethereal diazomethane until the $FeCl_3$ and 2:6-dichloroquinone chloroimide tests could no longer be obtained. The resultant glucuronide syrup was subsequently hydrolysed with 2N-HCl, and the liberated phenol isolated in the usual manner. The crystalline product obtained was identified as 4-hydroxy-2-methoxyacetophenone (IX), by comparison with a pure sample of the compound prepared synthetically; m.p. and mixed m.p. 137–138°; m.p. and mixed m.p. of the corresponding *p*-toluenesulphonate derivative, 121·5°. 2-Hydroxy-4-methoxyacetophenone (X) melts at 50°, and the corresponding *p*-toluenesulphonate derivative appears to be an oil.



(IV) (V) (VI)



(IX) (X)

The structure of resacetophenone glucuronide is therefore established as 4-(β-D-glucuronosido)-2-hydroxyacetophenone (V). Resacetophenone is thus behaving similarly to the dihydroxychlorobenzenes as regards the orientation of glucuronic acid conjugation. The hydroxyl group which is farthest away from the substituent group (Cl or $—COCH_3$) is preferentially conjugated.

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Organic Catalysts of the Oxidation of Ascorbic Acid and of Ketoses. By J. PRYDE. (*Physiology Institute, Newport Road, Cardiff*)

Two of the possible metabolites of 2:4:6-trinitrotoluene (T.N.T.), namely, 2:4:6-trinitrobenzoic acid and 2:4:6-trinitrobenzyl alcohol (Channon, Mills & Williams, 1944), catalyse the oxidation of ascorbic acid by molecular oxygen at pH 7·4, both in the presence and in the absence of surviving mammalian tissue. The oxidation has been followed by the Warburg manometric technique and by titration and colorimetric techniques (Musulin & King, 1936; Roe & Oesterling, 1944), both in the presence and in the absence of inhibitors of metallic ion catalysts such as sodium diethyl dithiocarbamate, 8-hydroxyquinoline and cyanide. The reaction catalysed is:

$$C_6H_8O_6 + O_2 = C_6H_6O_6 + H_2O_2,$$

since, in the presence of ox-liver catalase (impregnated filter-paper strips), the measured oxygen uptake is halved. The catalysis is a true organic one and therefore resembles that described by Snow & Zilva (1942), who observed the catalysis of ascorbic acid oxidation by tea infusions and by gallic acid, and whose observations on gallic acid have been confirmed in the course of the present investigations.

No catalytic effect has been observed with T.N.T., itself, nor with certain compounds derived from or closely related to T.N.T., for instance, 2:6-dinitro-4-hydroxylaminotoluene, 2:6-dinitro-*p*-toluidine, 2:4:6-trinitro-*m*-cresol and 4:6-dinitro-*o*-cresol. Various mono- and dinitrobenzoic acids, mononitrobenzyl alcohols, picric and picramic acids are likewise negative; so, too, are nicotinic acid, riboflavin and thiamin.

On the other hand, marked catalysis of ascorbic acid oxidation has been observed in the presence of sodium 1:2-naphthoquinone-4-sulphonate and of 1:4-naphthoquinone which themselves show only a negligible oxygen uptake at pH 7·4. As already stated T.N.T. itself has no catalytic effect but the isomeric 2:4:5-trinitrotoluene acts as a catalyst, an observation possibly related to its ease of hydrolysis to 2:4-dinitro-5-hydroxytoluene (Brownlie & Cumming, 1946). Another polynitro substance which acts as a catalyst is 1:2:3-trinitronaphthalene. It seems possible that a considerable number of organic compounds may act as catalysts of the aerobic oxidation of ascorbic acid.

Meanwhile, it has now been observed that the oxidation *in vitro* of the ketoses fructose and sorbose in alkaline phosphate media (pH 11·5) by molecular oxygen is also strongly catalysed by 2:4:6-trinitrobenzoic acid and by 2:4:6-trinitrobenzyl alcohol but not by gallic acid. Sodium 1:2-naphthoquinone-4-sulphonate, which itself shows but slight oxygen uptake at pH 11·5 in the medium used, gives marked catalysis of fructose oxidation. In the same alkaline phosphate media 2:4:6-trinitrobenzyl alcohol shows some catalytic effect on the rates of oxidation, much lower than those of the ketoses, shown by the aldoses glucose, mannose and galactose.

Work continues with the hope of elucidating the mechanism and possible significance of these catalytic effects.

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Kinetic Studies of the Metabolism of Foreign Organic Compounds. By H. G. Bray, W. V. Thorpe and K. White. (*Physiology Department, Medical School, University of Birmingham*)

Certain radicals in aromatic compounds undergo characteristic reactions in the body. Most of these reactions fall within the following classification:

(1) Conjugation.

(2) Conversion of potential to actual centres for conjugation.

(3) Introduction of new centres for conjugation.

Compounds containing such radicals have more than one possible fate, and investigations in this laboratory into the metabolism of these compounds have shown that in general, while more than one reaction occurs, conjugation is preferred to formation of conjugable groups, and conversion of potential centres to introduction of new ones (see Thorpe, 1950).

The relative amounts of metabolites and the time taken for excretion will depend on the velocities of these reactions. Consequently, a series of investigations has been planned in which it is hoped to determine the velocities of typical metabolic reactions which foreign organic compounds may undergo in the rabbit. In this first investigation the formation of benzoic acid from toluene, benzyl alcohol, benzaldehyde and benzamide and its conjugation with glycine and glucuronic acid have been studied.

It has been shown that all but the hippuric acid synthesis are first order reactions, for which the velocity constants have been determined. The rate of conjugation with glycine is constant. It had previously been observed that the ratio of glycine conjugate to glucuronide excreted varied with the compound fed and the dose level (Bray, Neale & Thorpe, 1946; Bray, Humphris & Thorpe, 1949). The present findings show that this ratio is a function of the velocity constants and the dose level. In addition the values of the velocity constants help to elucidate the mechanism of the oxidation of toluene.

The experimental method essentially comprised the analysis of blood samples, and of urine samples timed and collected by means of a fraction-collector.

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Further Observations on the Use of Paper Chromatography for the Detection of Phenolic Compounds in Metabolic Studies. By H. G. Bray, H. J. Lake, W. V. Thorpe and K. White. (*Physiology Department, Medical School, University of Birmingham*)

Methods for the detection by paper chromatography of the phenolic compounds encountered in metabolic studies of the hydroxybenzoic acids and amides (Bray, Thorpe & White, 1950*a*) and of cresols (Bray, Thorpe & White, 1950*b*) have already been described. In subsequent work the following solvent mixtures have proved of value: (1) light petroleum (b.p. 40–60°) saturated with formic acid (98–100%) for fast-moving compounds, (2) benzene saturated with formic acid which gives better separation than the benzene-acetic acid-water mixtures used previously, (3) chloroform-acetic acid-water mixtures for slow moving compounds. A mixture of *n*-butanol (4 vol.), pyridine (8 vol.), saturated aqueous NaCl (5 vol.), ammonia, sp.gr. 0·880 (3 vol.), gives better separation (e.g. of *o*-, *m*- and *p*-hydroxybenzoic acids) than the original mixture of Evans, Parr & Evans (1949). Preliminary experiments with columns of paper powder (cf. Hough, Jones & Wadman, 1949) have given encouraging results in the isolation of urinary phenols.

It has now been shown that the phenolic acid of normal rabbit urine previously assumed to be mainly *p*-hydroxybenzoic acid (Bray *et al.* 1950*a*) is a mixture of both *m*- and *p*-acids, the former predominating (cf. Lederer & Polonsky, 1948). The amount of the *m*-isomer is not significantly increased by administration of benzoic acid.

Both 4-amino-3-hydroxybenzenesulphonic acid and its amide have been identified as hydrolysis products of the glucuronide fraction of urine from rabbits dosed with sulphamezathine.

In earlier studies of the metabolism of amino- and nitro-benzamides (Bray, Lake, Neale, Thorpe & Wood, 1948; Bray, Thorpe & Wood, 1949) we were unable to identify by isolation all the hydroxylation products which would be expected theoretically. Paper chromatograms provide clear evidence of the presence of 3-amino-2-hydroxy-, 3-amino-4-hydroxy- and 3-amino-6-hydroxybenzoic acids in the hydrolysed urine of rabbits dosed with *m*-aminobenzamide, and of 2-amino-3-hydroxy- and 2-amino-5-hydroxybenzoic acids from rabbits dosed with *o*-nitrobenzamide. 2-Amino-5-hydroxybenzoic acid has also been detected as a metabolite of anthranilic acid.

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Spectrophotometric Determination of β-Glucuronidase Activity using *p*-Chlorophenylglucuronide as Substrate. By B. SPENCER and R. T. WILLIAMS. (*Department of Biochemistry, St Mary's Hospital Medical School, London, W.* 2)

p-Chlorophenylglucuronide (monohydrate; m.p. 151°, $[\alpha]_D - 82{\cdot}5°$ in water) is a compound which can be made biosynthetically in good yield (1·1 g./g. *p*-chlorophenol) by direct ether extraction of the 12 hr. urine of rabbits receiving *p*-chlorophenol orally (0·5 g./kg.). Furthermore, small amounts of *p*-chlorophenol can be easily determined as its anion with the Unicam spectrophotometer in the presence of relatively large amounts of *p*-chlorophenylglucuronide. The ultraviolet absorption spectra of *p*-chlorophenol and its glucuronide in acid and alkali are given in Table 1. It is clear from this table that, in alkali, *p*-chlorophenol has a totally different spectrum from its glucuronide and hence by measuring the extinction at 245 mμ. (or in strong solutions at 298 mμ.) *p*-chlorophenol can be estimated in the presence of its glucuronide. *p*-Chlorophenylglucuronide has only a small absorption at these wavelengths, i.e. at 245 mμ., ε is 70 and at 298 mμ., ε is *c.* 20. In the dilutions used in our experiments, 5 μg. of *p*-chlorophenol were readily estimated. The extinctions at 245 and 298 mμ. obey Beer's law and are not affected by the acetate buffer and ethanol used in the assay.

Table 1

	In 0·1 N-HCl		In 0·1 N-NaOH	
	mμ	$\epsilon_{max.}$	mμ.	$\epsilon_{max.}$
p-Chlorophenol	222	8,900	245	11,650
	280	1,600	298	2,600
p-Chlorophenyl-glucuronide	220	10,700	219·5	10,800
	275·5	910	276	970

The method was applied to an active β-glucuronidase extract prepared from ox-spleen according to Talalay, Fishman & Huggins (1946) and fractionated according to Mills (1948). For the estimation protein, which has a significant absorption at these wavelengths, was removed with ethanol (80 % final concentration).

Using this method pH optima were demonstrated at pH 4·1 and 5·2 in acetate buffer, and the activity of the enzyme was shown to be proportional to time and concentration of the enzyme. These and other results were discussed.

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An Ether-Soluble Precursor of Coproporphyrin in Urine. By D. N. RAINE (introduced by F. K. HERBERT). (*Department of Chemical Pathology, The Medical School, King's College, Newcastle upon Tyne*)

When freshly passed human urine is treated with acetic acid and extracted with ether, it is found that the ether extract contains both preformed coproporphyrin, which can be immediately extracted by 1 % HCl (w/v), and a residual non-fluorescent substance which yields fluorescent porphyrin when the ether extract is allowed to stand in daylight. The solubilities of this porphyrin are those of coproporphyrin. When the urine is passed in the dark, and handled in a dark room up to the stage of extraction of the preformed coproporphyrin with HCl, and the ether layer then exposed to daylight, the yield of preformed coproporphyrin is less, and the yield of total coproporphyrin greater, than when the pro-

cedure is carried out entirely in daylight. Any exposure of urine to light before extraction leads to losses. Normally the major fraction of urinary coproporphyrin is in the form of precursor.

The transformation of precursor to porphyrin has been studied with a view to defining the chemical reaction and developing a satisfactory quantitative method for the estimation of total coproporphyrin. Evidence has been obtained that the precursor is a reduced form of the porphyrin, possibly identical with the porphyrinogen obtained by Fischer & Orth (1937) by reduction of porphyrin.

The method of exposing the ether extract to light after removal of preformed coproporphyrin can be used for quantitative estimation of the precursor, but the time required for the conversion is 24 hr. in summer light, and 3–4 days in winter. Various oxidants have been employed to accelerate the conversion and the best is quinhydrone added to the ether extract in a final concentration of approximately 0·04%. In daylight with quinhydrone the maximum yield is obtained in 2 hr.

The standard values for normal coproporphyrin excretion in man require revision in the light of these findings. Preliminary estimations indicate a normal total urinary excretion of the order of 200 μg./day.

When freshly passed urine is treated by the adsorption technique of Sveinsson, Rimington & Barnes (1949), preformed coproporphyrin is adsorbed on the calcium phosphate precipitate and the precursor is not adsorbed.

The administration of ascorbic acid to normal subjects, to the point of saturation, leads to the excretion of an increased proportion of the precursor. This is in accord with the conception that the precursor is the reduced form of the porphyrin.

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The Distribution of Fructose in Fractionated Specimens of Human Semen. By J. Pryde. (*Physiology Institute, Newport Road, Cardiff*)

Opportunity has recently been afforded for the examination of a few total ejaculates of human semen which had each been collected in three fractions. Refractive index measurements, sperm counts and fructose determinations are quoted. Two specimens from the same donor gave the figures quoted under A 1 and A 2.

Fraction		Volume (ml.)	$n_D^{20°}$	Sperm (count/ml. $\times 10^6$)	Fructose (mg. %)
A 1	1	1·2	1·3456	650	120
	2	1·4	1·3496	205	530
	3	1·2	1·3540	75	906
A 2	1	1·0	1·3468	810	84
	2	1·3	1·3510	120	560
	3	1·2	1·3550	40	1016
B	1	0·8	1·3460	612	212
	2	1·2	1·3468	255	368
	3	2·5	1·3467	120	604

These figures show unusually sharp demarcation of the prostatic-spermatic and the vesicular components of the fluid (MacLeod & Hotchkiss, 1941; Gutman & Gutman, 1941) and strongly support the view that the fructose of human semen is provided by the seminal vesicles (Huggins & Johnson, 1933; Mann, 1946). Mann (1946) has commented on the variability of the fructose concentrations in semen of the same species. The fructose values for both fractions 3 of A 1 and A 2 suggest that, with strikingly high vesicular figures of the order here found, variations in the amount of the vesicular component included in the ejaculate could effect considerable fluctuations in the final fructose concentration of the whole semen.

The pattern above described, with its well-marked separation of components, cannot be regarded as of general occurrence, at least to the same degree. As an example, figures are quoted for specimen B from a different donor. In particular, the closeness of the three refractive index readings in B contrasts strongly with the considerable differences shown by the corresponding readings for A 1 and A 2.

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Studies on the Average Content of Nucleic Acids in Human Marrow Cells. By J. N. Davidson, I. Leslie and J. C. White. (*From the Department of Biochemistry, University of Glasgow, and the Department of Pathology, Postgraduate Medical School of London*)

In extension of previously reported analyses of the deoxyribonucleic acid phosphorus (DNAP) and ribonucleic acid phosphorus (RNAP) content of aspirated human bone marrow (Davidson, Leslie & White, 1947, 1948), we now report a modification involving enumeration of the nucleated cell content of the samples analysed. Results are expressed in terms of DNAP and RNAP per cell (Table 1), and are average values for the growing and adult cell populations of the analysed samples. The recent results of Vendrely & Vendrely (1948, 1949) and of Mirsky & Ris (1949) suggest a striking constancy in the DNAP content of normal cell nuclei from the tissues of any given species, and our figures for DNAP are of the same order as those quoted by the Vendrelys for human liver nuclei.

There is no significant difference between the means for the normal and the leukaemic series, either as a whole, or considering only acute leukaemia prior to therapy.

A small series of 6 cases of iron-deficiency anaemia has not shown significant variation of the mean DNAP and RNAP per cell from normal.

Results obtained from cases of pernicious and other megaloblastic anaemias are shown in Tables 2 and 3.

It must be noted clearly that the group under therapy cannot be considered as returned to normal, either as regards blood picture, marrow cytology or adequacy of therapy. The significant fall in RNAP from that in the group prior to therapy parallels the general increase in maturity of the marrow under therapy. Cases fully treated and returned to normal are under investigation.

Table 1

Normal human marrow

Values of Nucleic Acid Phosphorus (NAP) in $\mu g. \times 10^{-7}$ per cell

	DNAP 18 obs. on 16 individuals	RNAP 20 obs. on 18 individuals	Ratio RNAP/DNAP
Mean	8·54	6·33	0·75
S.E. of obs.	2·89	3·03	0·326
Observed range	4·0–15·0	2·1–13·5	0·43–1·9

Marrow from cases of leukaemia of various types, before and during therapy

	28 obs. on 15 cases	24 obs. on 12 cases	
Mean	8·75	7·59	0·90
S.E. of obs.	3·05	3·72	0·30
Observed range	3·9–17·4	2·6–17·4	0·3–1·8

Table 2. *Cases of pernicious anaemia and other megaloblastic anaemias*

NAP in $\mu g. \times 10^{-7}$ per cell

		DNAP 28 obs. on 12 cases	RNAP	Ratio DNAP/RNAP 28 obs. on 13 cases
Group as a whole	Mean	12·6	10·9	0·87
	S.E.	4·56	5·03	0·27
	Observed range	6·6–22·8	2·3–25·1	0·35–1·5
		12 obs. on 12 cases	11 obs. on 11 cases	12 obs. on 12 cases
Group prior to therapy	Mean	12·57	13·38	1·06
	S.E.	4·17	5·19	0·249
	Observed range	8·1–22·8	7·5–25·1	0·69–1·5
		17 obs. on 8 cases	15 obs. on 8 cases	16 obs. on 9 cases
Group during the course of therapy	Mean	12·63	9·09	0·73
	S.E.	4·36	4·21	0·198
	Observed range	6·6–18·8	2·3–17·6	0·35–1·0

Table 3. *t test of significance between means*

		DNAP	RNAP	Ratio RNAP/DNAP
Megaloblastic series as a whole compared with normal series	*P*	<0·001	<0·001	0·2–0·1
	Degrees of freedom	44 Highly significant	44 Highly significant	46 Not significant
Megaloblastic series before therapy compared with normal	*P*	0·01–0·001	<0·001	0·01–0·001
	Degrees of freedom	28 Highly significant	29 Highly significant	30 Highly significant
Megaloblastic series during therapy compared with normal	*P*	0·01–0·001	0·05–0·02	0·8–0·7
	Degrees of freedom	33 Highly significant	33 Significant	34 Not significant
Megaloblastic series before and during therapy compared	*P*	0·7–0·6	0·05–0·02	<0·001
	Degrees of freedom	27 Not significant	24 Significant	26 Highly significant

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Fluoroacetate Poisoning and 'Jamming' of the Tricarboxylic Acid Cycle; Mode of Action of an 'Active' Fluoro Compound Synthesized via this Cycle. By P. Buffa, W. D. Lotspeich, R. A. Peters and R. W. Wakelin. (*Department of Biochemistry, University of Oxford*)

So far no isolated enzyme has been inhibited by fluoroacetate. The hypothesis has been advanced by Liébecq & Peters (1949) (see also Martius, 1949) that the inhibition of citrate oxidation, occurring also *in vivo* (Buffa & Peters, 1949), is due to the 'jamming' effect of an enzymically synthesized fluorotricarboxylic acid in the Krebs tricarboxylic acid cycle. In support of this hypothesis, Buffa, Peters & Wakelin (1950) have isolated, from guinea-pig kidney homogenates treated with fluoroacetate, a tricarboxylic fraction, which is 'active' in preventing disappearance of added citrate. This active fraction is mainly citrate; it contains no fluoroacetate, but there is present a small amount of a F-compound which is chromatographically inseparable from the tricarboxylic acids.

We have tried to find the exact point of inhibition in the enzymes of the tricarboxylic acid cycle by determining the effect of the 'active' fractions upon aconitase (Johnson, 1939), isocitric dehydrogenase (Adler, Euler, Günther & Plass, 1939) and oxalosuccinic decarboxylase (Ochoa & Weiss-Tabori, 1948), obtained from rat and pig heart tissue. Tables 1, 2 and 3 show that the results were negative, even when amounts of 'active' fraction were used 80 times larger than those inhibiting citrate disappearance in the kidney homogenates.

All the evidence from experiments *in vivo* and *in vitro* (? mitochondrial homogenates) points to inhibition by the 'active' compound at either the aconitase or isocitric dehydrogenase stage. Hence, we are led to the conclusion that the complete system has properties not present in its isolated enzyme components. Whether these be due to factors of organization or to missing components must be decided by further work.

Table 1. *Rat heart aconitase*

	Citric acid (μmol.)	
Time (min.) ...	0	60
Additions:		
cis-Aconitate (5 μmol.)	0·21	3·90
cis-Aconitate + 'active' fraction	0·08	3·96
Citrate (5 μmol.)	4·90	4·34
Citrate + 'active' fraction	5·27	4·38

Table 2. *Pig heart isocitric dehydrogenase*

	$E_{340\,m\mu}$. (max. value)
DL-*iso*citrate only	0·076–0·065
Same + 'active' fraction	0·075
Same + *p*-chloromercuricbenzoic acid $1·33 \times 10^{-5}$ M	0·004

Table 3. *Pig heart oxalosuccinic decarboxylase*

(CO_2 evolution from 10 μmol. oxalosuccinate in 30 min. at 13·5° C. Net values)

	CO_2 (μl.)
Enzyme alone	83
Enzyme + 'active' fraction	76
Enzyme + DL-*iso*citrate (control)	14

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Metabolic Maintenance of the Inorganic and Creatine Phosphates of Brain Tissue *in vitro*. By H. McIlwain and J. D. Cheshire. (*Biochemical Laboratories, Institute of Psychiatry, Maudsley Hospital, London, S.E.* 5)

The phosphates of mammalian brain change markedly in concentration with changed activity of the central nervous system (for a recent assessing see McIlwain, 1950). Inorganic phosphate and creatine phosphate are among the constituents most sensitive to the actions of convulsants (Stone, Webster & Gurdjian, 1945; Klein & Olsen, 1947) and narcotics (Stone, 1940; Le Page, 1946). To investigate the actions of such substances we desired to study their actions on separated brain tissue. The inorganic and creatine phosphates of brain are, however, found to undergo extremely rapid change after death of animals. Fall in phosphocreatine to 30 % of the original value in 3 sec. has been observed in dogs (Kerr, 1935) and to 25 % in mice on decapitation (Stone, 1940). About a threefold increase in inorganic phosphate occurred concomitantly. This contrasts with observations on the balance of products of carbohydrate metabolism in brain slices and on the rate of such metabolism. Here the findings, some time after death, can be very close to those determined by arterial-venous difference (Schmidt, Kety & Pennes, 1945).

We have now observed in guinea-pig brain, a post-mortem fall in creatine phosphate and rise in inorganic phosphate comparable to those described above. However, we have found that after respiration of brain cortex slices in balanced salines with substrates, the phospho-creatine of the slices greatly increased, and their inorganic phosphate fell. With suitable mixtures, values approaching those normal to the tissue *in vivo* could be reached. In addition to glucose, glutamate markedly affected the values reached. The findings regarding the maintenance of the phosphates of brain tissue *in vitro* thus parallel those of Krebs & Eggleston (1949) regarding its maintenance of potassium salts. Phosphates were determined in our experiments after separation by Ca-ethanol precipitations. The creatine phosphate precipitated was estimated after conversion to creatinine; it is differentiated by the conditions of precipitation, from creatine present as such.

Estimation of brain phosphates has been applied in studying the action of narcotics. By determining the inorganic phosphate, phosphocreatine and lactic acid during *in vitro* metabolism by brain cortex, it has been shown (Buchel & McIlwain, 1950) that the inhibition of respiration associated with the action of narcotics *in vitro* (see for example Quastel & Wheatley, 1933; Quastel, 1943) has very different characteristics from that previously found (Stone, 1938, 1940; Richter & Dawson, 1948) to be associated with their action *in vivo*.

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The Biosynthesis of Choline Methyl Groups in the Rat. By H. R. V. Arnstein. (*The National Institute for Medical Research, Mill Hill, London, N.W.* 7)

After feeding ^{14}C-methyl-labelled choline to rats the isotope was found both in the respiratory CO_2 and in the β-carbon atom of serine (Sakami, 1949). Since the β-carbon atom of serine can arise by the *in vivo* condensation of formate and glycine (Sakami, 1948) it seems likely that the first step of the oxidation of choline methyl groups is the formation of a 1-carbon fragment, probably formate or formaldehyde.

Although the supply of labile methyl groups to the rat, either as choline or methionine, is believed to be essential, it seemed possible that the animal is able to synthesize a part of its requirement from other sources. In this connexion it was of interest to examine the reverse reaction, i.e. the reduction of formate and similar potential 1-carbon fragments to choline methyl groups.

The isotopically-labelled compounds listed in Table 1 were fed to adult rats kept on a stock diet. After a time interval of between 1 and 5 days the animals were killed. The choline was isolated as reineckate, converted to the chloroplatinate and degraded to trimethylamine chloroplatinate (du Vigneaud, Cohn, Chandler, Schenk & Simmonds, 1941). The results of these experiments are given in Table 2. All the compounds except carboxyl-labelled glycine gave rise to isotopically labelled choline, containing isotope in both the ethanolamine and methyl groups. It is concluded that the 1-carbon fragment derived from the β-carbon atom of L-serine and the α-carbon atom of glycine, as well as methanol and formate, can be converted to choline methyl groups by the intact rat. (A similar experiment with ^{14}C-methanol has recently been reported by du Vigneaud & Verly (1950). CO_2, on the other hand, is not reduced, as carboxyl-labelled glycine was not a precursor of choline methyl groups. These results further show that the conversion of glycine to choline, which has previously been demonstrated with ^{15}N by Stetten (1941, 1942), takes place through serine with loss of the carboxyl group.

Table 1

Exp. no.	No. of rats	Body wt. (g.)	No. of days kept before killing	Labelled compound	Wt. fed (mg.)	Radioactivity (μC.) or atom % excess ^{15}N
1	2	508	2½	D-β-^{14}C-serine	142·6	11·7
2	2	550	2½	L-β-^{14}C-serine	122·0	10·0
3	1	302	3	α-^{14}C-glycine	9·6	9·6
4	1	260	1	^{14}C-methanol	13·7	10·3
				^{15}N-glycine	10·0	30·2
5	1	387	3	^{14}C-sodium formate	41·7	22·3
				^{15}N-glycine	10·0	30·2
6	2	590	4	Carboxyl-^{14}C-glycine	45·6	20·5
				^{15}N-glycine	53·0	30·2

Table 2

Exp. no.	Labelled compound fed	Radioactivity (10^{-3} μC./mM.)		Dilution factor*		% radioactivity in trimethylamine
		Choline	Trimethylamine	Choline	Trimethylamine	
1	D-β-^{14}C-serine	Inactive	—	—	—	—
2	L-β-^{14}C-serine	9·89	2·08	184	878	21·0
3	α-^{14}C-glycine	9·31	1·70	341	1866	18·2
4	^{14}C-methanol	13·86	5·52	286	719	39·8
	^{15}N-glycine	—	—	172	—	—
5	^{14}C-sodium formate	22·66	4·52	254	1278	19·9
	^{15}N-glycine	—	—	174	—	—
6	Carboxyl-^{14}C-glycine	Inactive	—	—	—	—
	^{15}N-glycine	—	—	302	—	—

* The dilution factor given is the reciprocal of the 'concentration coefficient' as defined by Anker (1948). It gives the dilution of isotope which would have been found if exactly 1 mM./100 g. body wt. had been fed.

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The Effects of Alcohols on Cholinesterases. 1. General. By K. P. Fellowes, J. P. Rutland and A. Todrick

Alcohols affect the hydrolysis of 0·018 M-acetylcholine by rat brain in two different ways. At low concentrations they cause activation but above an optimum concentration the activation rapidly falls off and with increasing alcohol concentration complete inactivation occurs.

The optimum concentration is approximately halv d for each additional carbon atom in the alcohol. The degree of activation is maximal with *n*-butanol and falls off rapidly on either side.

n-Butanol has been studied in some detail. The activation appears to be almost instantaneous and is completely reversible by dialysis. The inactivation is progressive and appears to have a high temperature coefficient. It is not reversible by dialysis. The alcohol concentrations involved are probably sufficiently high for the latter effect to be due to denaturation.

The effect of *n*-butanol on the activity of different enzyme preparations against the three standard substrates has been examined. With one exception all show activation:

Enzyme	Substrate	Maximum activation (%)
Rat brain	Acetylcholine (0·018 M)	110
Human erythrocyte	Acetylcholine (0·018 M)	55
Horse serum	Benzoylcholine (0·006 M)	28
Rat brain	Acetyl β-methylcholine (0·028 M)	14

The optimum activating concentrations are close to 0·2 M (somewhat higher with the brain-acetylcholine system).

The hydrolysis of acetylcholine by serum cholinesterase is inhibited by butanol. The inhibition occurs in the concentration range in which the other systems are activated and increases progressively with butanol concentration.

The Effect of Alcohols on Cholinesterases. 2. Investigation of the Mechanism of Activation. By K. P. Fellowes, J. P. Rutland and A. Todrick

The results quoted in the previous paper and, in particular, the exceptional behaviour of the serum cholinesterase-acetylcholine system have suggested a cause for the activation. According to Augustinsson (1948) this latter system is the only one of the five examined in which inhibition of the enzyme by excess substrate does not occur. The theory is advanced that alcohols interfere with the inhibition by excess substrate, the net result being an activation.

This has been tested by varying the substrate concentration. If the hypothesis is correct, the activation should disappear at substrate concentrations below the optimum.

The inhibition of the serum cholinesterase-acetylcholine system has been found to obey the equation of Lineweaver & Burk (1934) for reversible competitive inhibition though there is some deviation at the lowest concentrations.

The optimum substrate concentrations for the serum cholinesterase-benzoylcholine and brain cholinesterase-acetylcholine systems are low and there are considerable practical difficulties in working below them. However, in the latter case, it has been possible to demonstrate a shift in the optimum from pS 2·6 to 2·35 due to 0·19 M-*n*-butanol and a falling off in activation from 65 % at pS 2·0 to a negligible value at pS 2·9.

The system brain cholinesterase-acetyl β-methylcholine is much more satisfactory to work with since it has a pS optimum of 1·8–2·0. It has been possible to show that *n*-butanol causes 20–30 % activation at pS 1·0–1·5 but that at pS 2·4 and 2·7 there is an inhibition of the same value.

This result is in accordance with the hypothesis advanced. Augustinsson (1949) has already suggested that the inhibition caused by a competitive reversible inhibitor will depend on the substrate concentration. However, it does not appear to have been suggested before that the use of super-optimal substrate concentrations could result in inhibitors bringing about apparent activations.

The use of such high substrate concentrations has been not uncommon. It is not suggested that this will have been a major source of error except where competitive reversible inhibitors are involved.

We are indebted to the Chief Scientist, Ministry of Supply, for permission to publish these results.

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The Turn-over Number of Cholinesterases. By W. K. Berry (introduced by D. R. Davies)

The turn-over number (T.N.) of cholinesterase (ChE) cannot be expressed in terms of molecules of enzyme because samples pure enough for molecular weight determinations are generally not available. A T.N. in terms of active centres can be obtained comparatively easily if certain assumptions are made, for all of which there is some experimental support. These are that:

(1) there is a bimolecular reaction between active centres and an inhibitor,

(2) inhibition is irreversible,

(3) the inhibitor reacts practically exclusively with ChE.

When the inhibition reaction is allowed to go to completion, the loss in enzyme activity is directly proportional to the quantity of inhibitor added.

Dicyclohexyl fluorophosphate (DCFP) and diethyl *p*-nitrophenyl phosphate (E 600), both potent inhibitors of ChE, have been used because they are extremely resistant to hydrolysis. A fresh sample of E 600 was used as a check on the DCFP, which was over a year old. Both gave the same T.N. with partially purified human erythrocyte ChE, viz. 162,000 molecules ACh per minute. This was taken as evidence that the DCFP had not deteriorated. It is the better inhibitor because it is more stable and reacts more rapidly.

A comparison of crude and 30-fold purified human erythrocyte ChE showed that the probability that the two estimations were identical was 0·3. This finding, that a good value of T.N. could be obtained from unpurified material, enabled a species survey to be carried out without the necessity of purifying each sample. It would nevertheless apply only to such tissues as can be shown to contain predominantly or exclusively one ChE.

The following provisional results are given for the blood of different species:

Species	Plasma	Cells
Human	48,000	171,000
Horse	94,000*	13,000
Goat	—	53,000*
Dog	99,000	50,000
Rat	—	10,000

* Partially purified.

In addition to differences in T.N., there were some differences in the rate of inhibition, that of dog cells being slowest.

It is not yet possible to say whether the species differences observed are due to the existence of different enzymes, or to differences in the environment of the same basic types.

I am indebted to the Chief Scientist, Ministry of Supply, for permission to publish this communication.

The Variation of the Cholinesterases of the Blood in Health. By D. R. Davies and J. P. Rutland

Lack of adequate knowledge of the variation of blood constituents in health is a major deficiency in clinical biochemistry. The main reason for this lies in the difficulty, not only in obtaining a sufficient number of blood specimens from healthy people but also in obtaining adequate social and medical data, for the problem is as much statistical and demographic as it is analytical. In this communication the variation in both the red cell and plasma cholinesterases of approximately 200 healthy adults between the ages of 18 and 74 is reported. Three groups were investigated:

(i) A Service group drawn from the R.N., Army and R.A.F., consisting of 72 males between 18 and 30 concerning whom documentary evidence of their standard of physical fitness was forthcoming. They were all of the highest standard of fitness.

(ii) A group of 72 males between 18 and 64 drawn from the general population of south-west England. These were all blood donors and none had any recent history of serious illness.

(iii) A group of 53 females from the same source, aged 25–74.

The mean red cell ChE of the Service group was significantly higher than that of either the male or female groups, between which there was no difference. This difference was not due to age, for there was no difference in either the R.B.C. or plasma enzyme with age. A more detailed analysis suggested that possibly the R.B.C. enzyme did in some way reflect the state of fitness of the individual.

The R.B.C. enzyme varies 30% about the mean value (this is based upon the usual 95% fiducial limits), and the plasma enzyme 40%.

Technique	Cells: No. in group	Cells: Coeff. of variation	Plasma: No. in group	Plasma: Coeff. of variation
Warburg; acetylcholine as substrate	188	15·7	180	21·2
Warburg; acetyl-β-methylcholine as substrate	50	15·4	—	—
Warburg; benzoylcholine as substrate	—	—	49	25·8
Electrometric	48	15·9	47	24·8

The coefficient of variation, i.e. the percentage standard deviation, is a valuable parameter for measuring the spread of normal (healthy) levels around the mean, for it is an index whose value is independent of the technique of measuring the enzyme. Three methods were used for determining ChE activity of various specimens, and the coefficients of variation were determined. They were essentially the same, irrespective of the method.

The slightly wider values in the case of the plasma may be due to the fact that the plasma is less homogeneous with reference to its ChE content.

We wish to thank the Chief Scientist, Ministry of Supply, for permission to publish this communication.

The Electrometric Method of Michel for the Estimation of Cholinesterases. By D. R. Davies and J. P. Rutland

It is often necessary in routine investigations to perform cholinesterase estimations in large numbers in a limited period of time. Hitherto, this has been done either by continuous titration or manometrically by Ammon's modification of the Warburg technique. Both these methods are time-consuming and unsuitable for the above type of work.

Recently Michel (1949) described an electrometric method for the estimation of ChE. This consists essentially of a measurement of the rate of fall of pH in weakly buffered solutions, consequent upon the accumulation of acid due to the enzymic hydrolysis of an appropriate choline ester. Since the only apparatus required was a pH meter, a constant temperature bath and suitable test-tubes, and the only observations needed were a limited number of pH determinations, the method seemed particularly suitable for the performance of large numbers of estimations.

The original paper is deficient in experimental proof of a number of issues upon which the method depends, so we have carried out experiments to examine these points. The principle of the electrometric method is that the fall in pH should be directly proportional to the time over a suitably wide range of pH. Michel implies that this condition is sensibly satisfied under the conditions of the estimation, but adduces no data to substantiate it. Furthermore, he himself worked with human red cells and serum only, although later workers in the same laboratories have quoted results using goat and dog bloods, and also for brain. We have followed the fall in pH using R.B.C. and plasma of various species and homogenates of rat tissues (see Fig. 1).

We have also determined the relationship between enzyme concentration and activity in goat cells diluted with water and buffer and also plasma diluted with inactivated plasma. Provided the activity was not less than 0·1 Δ pH/hr. (Michel defines the unit of enzyme activity as the rate of fall of pH/hr., i.e. Δ pH/hr.), then a strict proportionality between enzyme concentration and activity was observed.

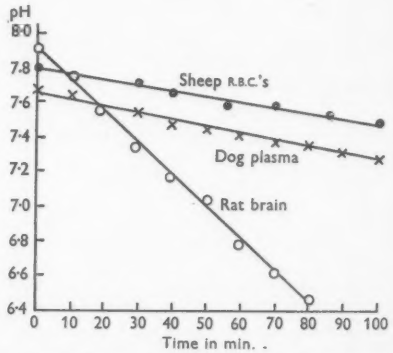

Fig. 1. The variation of pH with time due to cholinesterase in a barbiturate buffered system with acetylcholine as substrate. Similar curves have been obtained for:

Plasma	R.B.C.	Tissues (rat)
Rabbit	Rabbit	Liver
Dogs	Dogs	Heart
Goat	Goat	
Human	Human	
Rat	Sheep	

Dilution = 1–50.

Correlation between the electrometric and Warburg methods, which was tested not only upon diluted enzyme preparations but also upon various specimens of human cells and plasma, was good.

This method is quite satisfactory for many problems involving the routine estimation of ChE in

R.B.C.'s, plasma and tissues, provided that such tissues contain reasonable amounts of enzyme, i.e. it is not suitable for estimating the very small amounts which may remain after the administration of anti-ChE drugs. In fairness, however, it should be pointed out that it is no worse than the manometric method in this respect.

We wish to thank the Chief Scientist, Ministry of Supply, for permission to publish this communication.

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DEMONSTRATIONS

A Bridge Unit for Filter-Paper Chromatography. By E. KAWERAU. (*Department of Chemical Pathology, St Mary's Hospital, Paddington, London, W. 2*)

The novel feature of this all-glass unit for filter-paper chromatography is the arrangement of the strip in the form of a bridge from one flask to another by passing it through a glass sleeve. The latter is made in two sections for ease of assembly and cleaning. All parts join by standard ground glass joints, the whole unit being made to the author's specifications by Messrs Quickfit and Quartz, Stone, Staffordshire. The overall length of the apparatus is 50 cm.; it can be accommodated in the incubator or the refrigerator as the analytical procedure may demand.

The ground-glass joint in the centre of the sleeve carries two holes to locate with each other, and by rotation it becomes possible either to admit or to exclude air from the apparatus. It has thus been made possible to study the effects of controlled evaporation on band and solvent movement along the filter-paper strip.

The *closed* unit. Reliable R_F values have been obtained for many sugars and they are identical with those obtained by either ascending or descending chromatography employing standard methods; it appears, therefore, that the change in the position of the filter paper in no significant way affects R_F values. Preliminary work on amino-acids and urinary pigments (porphyrins) tends to support this statement.

The *open* unit. This is a hitherto unexplored field of chromatography both from the theoretical and practical aspect. The technique employed at present is to let the filter-paper strip form a complete bridge between two solutions of different composition, one of them containing the substance under investigation. Somewhere in the centre of the strip the two solutions meet and a certain amount of mixing takes place, depending on the composition of the respective solvents. Evaporation provides the driving force for continuation of solvent movement after the paper has become saturated. Where a mixture of pigments is being analysed, they tend to appear in separate zones in which they become gradually more and more concentrated as the supply is being maintained. This method has been found useful in the analysis of urines containing small amounts of pathological pigments, and it should also have a place in preparatory work since it produces concentration as well as separation of the solutes if suitable solvents are chosen.

The advantages of the Bridge unit over existing apparatus are: all-glass construction, small size, interchangeable parts, ease of temperature control, ease of cleaning and the small gas volume which is released when the unit is opened for the removal of the filter-paper strip.

An Automatic Fraction-cutter for Use in Partition Chromatography. By K. S. DODGSON, J. PRYDE and A. L. SIMS. (*Physiology Institute, Newport Road, Cardiff*)

A unit which is capable of collecting automatically fifty samples at intervals ranging from 1 to 30 min. has been constructed at very low cost. The mechanism is controlled by a variable impulse timer, which has been built from a surplus R.A.F. aerial photography unit. There is no direct mechanical connexion between the motor driving the turntable and the turntable itself, so that the latter can be freely rotated for purposes of adjustment.

The mechanism (Sims, 1938) responsible for moving the turntable has been so designed that additional and larger turntables can be driven simultaneously by the same motor. The apparatus is provided with an automatic stop which can be made to operate at any predetermined time up to 24 hr.

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FORTHCOMING PAPERS

It is hoped to publish the following papers in the next issue of the *Biochemical Journal*:

Ayfivin and bacitracin: resolution of crude products into similar series of peptides. By G. G. F. Newton and E. P. Abraham

The biochemistry of bacterial toxins:

4. The lecithinase activity of *Clostridium haemolyticum* toxin. By M. G. Macfarlane

5. Variation in haemolytic activity of immunologically distinct lecithinase towards erythrocytes from different species. By M. G. Macfarlane

Studies in detoxication:

33. The metabolism of halogenobenzenes. A comparison of the glucuronic acid, ethereal sulphate and mercapturic acid conjugations of chloro-, bromo- and iodo-benzene and of the *o*-, *m*- and *p*-chlorophenols. Biosynthesis of *o*-, *m*- and *p*-chlorophenylglucuronides. By B. Spencer and R. T. Williams

34. The metabolism of chlorobenzene in the rabbit. Isolation of dihydrodihydroxychlorobenzene, *p*-chlorophenylglucuronide, 4-chlorocatechol glucuronide and *p*-chlorophenylmercapturic acid. By J. N. Smith, B. Spencer and R. T. Williams

The fate of certain organic acids and amides in the rabbit. 11. Further observations on the hydrolysis of amides by tissue extracts. By H. G. Bray, S. P. James, W. V. Thorpe and M. R. Wasdell

Determination of antrycide. By A. Spinks

The influence of scurvy and fall in weight in young guinea pigs on the alkaline phosphatase content of the serum and the zones of provisional calcification. By H. R. Perkins and S. S. Zilva

Chemical studies of peripheral nerve during Wallerian degeneration. 2. Lipids after nerve crush (axonotmesis). By N. S. Burt, A. R. McNabb and R. J. Rossiter

The interaction of serum albumins with calcium. By N. H. Martin and D. J. Perkins

The haemolytic acid present in horse brain:

1. Purification and identification as *cis*-octadec-11-enoic acid. By I. D. Morton and A. R. Todd

2. Examination by the insoluble monolayer technique. By E. D. Goddard and A. E. Alexander
Appendix: X-ray examination of the haemolytic fatty acid. By E. D. Goddard and M. F. Perutz

The composition of horse bot fly (*Gastrophilus intestinalis*) larva blood. By L. Levenbook

An outer metabolic region of the yeast cell. By E. J. Conway and M. Downey

pH values of the yeast cell. By E. J. Conway and M. Downey

Biological production of acid and alkali:

1. Quantitative relations of succinic and carbonic acids to the potassium and hydrogen-ion exchange in fermenting yeast. By E. J. Conway and T. G. Brady

2. A redox theory for the process in yeast with application to the production of gastric acidity. By E. J. Conway, T. G. Brady and E. Carton

The use of radioactive isotopes in immunological investigations:

3. Further studies on the fate of injected ^{32}P-containing proteins. By T. E. Banks, G. E. Francis, K. J. Franklin and A. Wormall

4. Antibodies to phospholipins. By G. E. Francis and A. Wormall

[*The Editors of the Biochemical Journal accept no responsibility for the Reports of the Proceedings of the Society. Abstracts of papers read are published as received from the authors.*]

PROCEEDINGS OF THE BIOCHEMICAL SOCIETY

The **287th Meeting** *of the Biochemical Society was held in the School of Biochemistry, Tennis Court Road, Cambridge, on Saturday,* 17 *June* 1950, *when the following papers were read:*

COMMUNICATIONS

The Effect of Dehydration by Hypertonic Saline on the Water Content of a Number of Organs in the Rat.

By R. A. McCance and J. R. Robinson. (*Department of Experimental Medicine, Medical Research Council, and University of Cambridge*)

There are indications that the water balance of mammalian tissues is not determined only by the osmotic pressure of the extracellular fluids. Thus Kudo (1921) subjected albino rats to acute and chronic thirst, and found that the organs did not lose weight to the same extent. McCance (1937) found that human erythrocytes did not increase in size during a deficiency of salt which lowered the osmotic pressure of the plasma electrolytes by about 10%. Gömöri & Frenreisz (1937) produced experimental pyloric stenosis in cats, and found that the osmotic pressure of the tissues (measured cryoscopically) increased despite a considerable fall in serum chloride.

In the present experiments dehydration was induced in normal adult rats by administering 35 ml. of 10% NaCl/kg. of body weight in single or in divided doses by stomach tube. This depleted the body water by diuresis, and at the same time increased the osmotic pressure of the extracellular fluids. Control animals were given corresponding amounts of distilled water. Next day the rats were killed, samples of blood and of a number of tissues were taken, and the amount of water in these was determined by drying them to constant weight at 105°. The pieces of tissue were blotted to remove the superficial blood before being placed in the tared tubes.

Table 1 shows the changes produced by dehydration in the percentage of water in the blood and tissues.

Table 1. *Percentage of total water*

Tissue	No. of samples	Seven control rats %	S.D.	Seven dehydrated rats %	S.D.	Difference t	P
Blood	6	79·1	±1·4	77·4	±1·4	1·7	1·91 0·1
Brain	14	77·5	±0·6	76·9	±0·8	0·6	2·23 <0·05
Kidney	14	75·5	±0·9	75·1	±1·0	0·4	1·17 0·2–0·3
Liver	14	69·2	±1·0	67·9	±1·1	1·3	3·22 <0·002
Leg muscles	9	75·9	±0·6	72·9	±0·9	3·0	7·8 <0·00001
Tongue	5	79·5	±0·5	76·9	±1·2	2·6	3·95 <0·01

The results show clearly that although the extracellular osmotic pressure must have increased to the same extent in all tissues, yet no two lost the same amount of water. The kidney, which was presumably reabsorbing a more dilute fluid than the glomerular filtrate under the influence of the antidiuretic hormone, lost practically none. The brain lost less water than the liver or the blood, and the leg muscles and tongue considerably more than any of the other organs.

It is possible that the differences were due to changes in the volumes of the extracellular compartments of the tissues, but the results are equally consistent with the view that the water content of cells is influenced as much by their metabolism as by the osmotic pressure of the extracellular fluid which bathes them (Robinson, 1950).

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Glutamic Acid Accumulation and Protein Synthesis in *Staphylococcus aureus*. By E. F. GALE. (*Medical Research Council Unit for Chemical Microbiology, University of Cambridge*)

When washed suspensions of *Staphylococcus aureus*, rendered deficient in internal free glutamate by growth in a minimal medium, are incubated with glutamic acid in the presence of glucose, the amino-acid passes into the cell and becomes highly concentrated therein as free glutamate (Gale, 1948). The process will only occur when energy is supplied by exergonic metabolism such as glucose fermentation. Metabolism leading to disappearance of some of the free glutamate within the cell takes place during incubation, but no protein or glutamyl-peptide formation can be shown (Gale & Mitchell, 1947). If a mixture of other amino-acids is added during the incubation, the accumulation of free glutamate within the cell ceases and protein synthesis occurs. A measure of the protein synthesis is given by determining the increase in the amount of glutamic acid liberated from the cells by acid hydrolysis; under optimal conditions the combined glutamate of the cells may increase by 30 % during 1 hr. incubation at 37° in the presence of glucose and a synthetic mixture of twenty natural amino-acids.

It has previously been assumed that the passage of glutamic acid into the cell, and its concentration therein, occurred prior to its condensation into peptide bonds. If this were the case, the rate of uptake of glutamate from the external medium should be approximately the same whether protein synthesis were occurring or not. It has been found experimentally that the uptake of glutamate from the medium is markedly reduced by the occurrence of protein synthesis. For example, the incubation of 100 mg. dry weight of cells at 37° for 1 hr. in a glutamate-glucose-buffer system results in the removal of 48·0 μmol. glutamate from the supernatant medium, of which approx. 60 % appears as free glutamate within the cells, the remainder being metabolized without any increase in combined glutamate. The addition, in a parallel experiment, of twenty amino-acids to the external medium resulted in a reduction of the amount of glutamate removed from the medium to 6·8 μmol.; during the same period the internal free glutamate of the cells was *reduced* by 6·7 μmol. and the combined glutamate of the cells increased by 11·0 μmol.

Experiments with varying concentrations of the amino-acid mixture show that the transfer and accumulation of free glutamate within the cell cease when protein synthesis occurs. It appears that glutamic acid either combines with other amino-acids to form cell protein or, in the absence of other amino-acids, passes into the cell and accumulates there as the free amino-acid. The former process inhibits the latter, and it is suggested that the initial metabolic stage involved in the energy-linked transfer of glutamic acid into the cell must also be an initial stage in the condensation of glutamic acid into peptide bonds.

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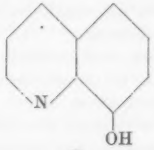
Effect of Inhibitors and Antibiotics on Glutamic Acid Accumulation and on Protein Synthesis in *Staphylococcus aureus*. By E. F. GALE and T. F. PAINE. (*Medical Research Council Unit for Chemical Microbiology, University of Cambridge*)

If the energy-linked passage of glutamic acid into *Staphylococcus aureus* occurs as a result of a metabolic process involving glutamic acid, it should be possible to inhibit this process without affecting the fermentation which provides the energy. Sodium fluoride or iodoacetate inhibit glucose fermentation to the same extent as they inhibit the accumulation of free glutamate within the cell. Sodium azide, 2:4-dinitrophenol and 8-hydroxyquinoline inhibit the accumulation of glutamate at concentrations markedly less than those required to inhibit fermentation.

The action of these inhibitors has also been tested on the rate of condensation of glutamic acid into cell protein (see preceding abstract). Sodium azide and 2:4-dinitrophenol inhibit protein synthesis and glutamate accumulation to significantly the same extent. 8-Hydroxyquinoline is a more effective inhibitor of glutamate accumulation than of protein synthesis. Aureomycin and chloramphenicol (chloromycetin) completely inhibit protein synthesis at limiting bactericidal concentrations, but have little effect on glutamate accumulation; 0·003 mM-chloramphenicol (1 μg./ml.) inhibits protein synthesis by 76 %, whereas 1·5 mM gives only 10 % inhibition of glutamate accumulation. Streptomycin at concentrations less than 0·1 mM is inactive in either system.

The Metal-Binding Properties of Riboflavin. By A. ALBERT. (*The Australian National University, 183 Euston Road, London, N.W. 1*)

Riboflavin has a hydroxyl-group *peri* to a ring nitrogen, a type of structure (cf. I) associated with metal-binding properties and found also in 8-hydroxyquinoline, in the naturally occurring pteridines and in a number of purines such as guanosine, all of which have a marked avidity for the divalent ions of heavy metals.



(I)

Potentiometric titration of riboflavin (0·001M) and metallic salts (0·0005M) with potassium hydroxide (under N_2, cf. Albert, 1950*a–c*) gave consistent values for K' (the stability constant of the 1:1 complex). However, in all but one case, further addition of alkali precipitated the 2:1 riboflavin:metal complex so that values for K'' could not be obtained. However, K_s (the overall stability constant) can be calculated approximately if it is assumed to lie between the customary limits:

$\log K_s$ lies between $(2 \log K')$ and $(2 \log K' - 1)$.

The values of $\log K'$ for riboflavin are shown in Table 1 together with those for glycine (typical of the divalent amino-acids) and 8-hydroxyquinoline (which is a structural analogue of riboflavin). The metals are arranged in the order of preference which complexing agents commonly exert for them (Mellor & Maley, 1948). It will be seen that this order is observed by glycine and, with one exception, by 8-hydroxyquinoline. Riboflavin, however, shows an unusual sequence: iron is preferred to all other metals, whereas nickel and cobalt are displaced below cadmium. Because the three substances listed in Table 1 have almost identical pK values, their ability to compete with one another for a trace of some metallic ion depends solely upon the magnitude of the stability constants.

These results open up the possibility that the activity of some riboflavin-containing enzymes may be associated with metallic ions, and the next stage should be to see what inhibitions can be effected by complexing agents. Xanthine oxidase is interesting in this respect because its substrate (xanthine), its product (uric acid), its coenzyme (riboflavin derivative) and the pteridines that inhibit it so strongly all have the metal-binding *peri*-hydroxyl structure (I). Already one example is known where riboflavin and iron are associated in nature. This is in transferrin (also known as siderophyllin and conalbumin), a β-globulin found in the white of egg and in the human blood stream (Bain & Deutsch, 1948).

Table 1

Substance	Log K' values for divalent metallic complexes							pK_a
	Cu	Ni	Zn	Co	Cd	Fe	Mn	
Riboflavin	6·6	4·1	5·6	3·9	4·7	7·1	3·4	9·93
Glycine	8·5	6·1	5·3	5·1	4·6	4·3	3·2	9·86
8-Hydroxyquinoline	12·2	9·85	*	9·1	7·2	8·0	6·8	9·89

* Too insoluble to determine.

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Effect of Certain Treatments on the Diabetogenic Activity of Growth Hormone Preparations. By E. REID (Beit Memorial Fellow). (*School of Biochemistry, University of Cambridge*)

Examination of a number of ox anterior pituitary preparations revealed a general parallelism between diabetogenic potency in the cat and growth-promoting potency in the rat, and it was subsequently shown that highly purified growth hormone preparations were diabetogenic (Cotes, Reid & Young, 1949*a*, *b*). This approximate parallelism is now being investigated more closely, with particular reference to the observation of Marks & Young (1940) that a crude (non-sterile) alkaline saline extract may lose

diabetogenic activity, but not growth-promoting activity, on incubation. Assay of one preparation against a second furnishes their 'D/G ratio'—the ratio of their relative diabetogenic potency (Reid, 1949) to their relative growth-promoting potency (Cotes *et al.* 1949*a*).

'*Untreated*' *preparations.* Several sterile crude extracts were compared with reference preparations, e.g. growth hormone prepared according to Wilhelmi, Fishman & Russell, 1948, which had a D/G ratio of approximately unity when assayed against one another. In some instances the D/G ratio was significantly higher than unity. The ratio tended to be inversely related to the time taken for extraction and Seitz-filtration.

'*Marks & Young*' *preparations.* Incubation of crude extracts before Seitz-filtration gave somewhat variable results—a decrease in diabetogenic potency with or without a decrease in growth-promoting potency. In at least one instance the D/G ratio, relative to growth hormone, was significantly lower than unity. Procedures for the isolation of growth hormone were applied to three incubated extracts, but the D/G ratios for the fractions thus obtained, relative to purified growth hormone, were in no instance lower than unity.

Preparations subjected to hydrolytic treatment. Prof. K. Linderstrøm-Lang kindly made available an enzyme, obtained from *B. subtilis*, which is known to hydrolyse specific bonds in the ovalbumin molecule (Linderstrøm-Lang & Ottesen, 1949). The diabetogenic potency of crude pituitary extracts and of purified growth hormone preparations decreased on incubation with this enzyme, but the growth-promoting potency diminished in parallel. When purified growth hormone was subjected to mild acid hydrolysis (incubation at pH 2·8), the D/G ratio was again unaltered.

It is possible that *crude* extracts contain a factor which, according to its concentration (which might be reduced by incubation before Seitz filtration), either augments or reduces the diabetogenic action of the growth hormone in the extracts. Such a factor might have a converse influence on the growth-promoting action of growth hormone. However, conclusive evidence of the cause of variations in D/G ratios is still lacking. It has still to be rigorously proved that the diabetogenic action of 'pure' growth hormone is an attribute of the growth hormone molecule itself, rather than of some factor which associates with growth hormone during fractionation procedures.

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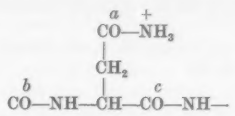
The Liberation of Free Amino-acids during the Acid Hydrolysis of Wool. By S. Blackburn. (*Wool Industries Research Association, 'Torridon', Headingley, Leeds* 6)

The isolation of peptides of glutamic and aspartic acids from wool following partial hydrolysis with concentrated HCl at 37° (Consden, Gordon & Martin, 1949) shows that free aspartic acid is not preferentially liberated as it is in the hydrolysis of proteins with weak acids (Partridge & Davis, 1950). It has now been found that the order in which the amino-acids are liberated from wool during HCl hydrolysis depends on the acid concentration and temperature of hydrolysis.

Hydrolysis with 0·1 N-HCl at the boil liberates free aspartic acid preferentially, as in the weak-acid hydrolysis of wool. The preferential liberation of aspartic acid is therefore not, as suggested by Partridge, a unique property of weak acids, but is dependent principally on the hydrogen-ion concentration in solution. As the acid concentration is increased, other amino-acids are liberated at increasing rates; and during short periods of hydrolysis aspartic acid is no longer the most abundant amino-acid to be seen on the chromatogram. Glycine is liberated fairly readily. Under the conditions of Consden *et al.* (1949) little free aspartic or glutamic acid is liberated in the early stages, low molecular weight peptides being produced. The effect of temperature has not yet been fully investigated. Thus the experimental results so far obtained may be summarized as follows: as the hydrogen-ion concentration is increased the amount of aspartic acid relative to the amount of other free amino-acids decreases.

It is considered that the following mechanism will explain this. When low concentrations of acid are employed, the protons combine with asparagine side chains. This charged group is then particularly

effective as a source of protons to hydrolyse peptide linkages *b* and *c* uniting asparagine in the chain, possibly because the NH_3^+ group is near in space to these peptide linkages. Under weak-acid conditions the rate of hydrolysis of amide groups is comparatively slow and occurs at a rate comparable to the splitting of the peptide linkages *b* and *c*. Free aspartic acid is thus liberated. When the acid concentration is raised the rate of hydrolysis of amide nitrogen becomes greater, removing the most effective source of protons for hydrolysing the peptide linkages *b* and *c*, and the preferential liberation of aspartic acid rapidly decays. The liberation of free glutamic acid shows much less variation with varying hydrogen-ion concentration than does the



liberation of free aspartic acid, possibly because the charged NH_3^+ group is more remote from the peptide linkage.

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Inhibition of Succinoxidase Activity of Mitochondria by *Clostridium welchii* toxin. By M. G. MACFARLANE. (*Lister Institute, Elstree*)

Wooldridge & Higginbottom (1938) found that *Clostridium welchii* α-toxin inhibited the aerobic oxidation of succinate by minced tissues, though succinic dehydrogenase and cytochrome oxidase tested separately were apparently not affected. *Cl. welchii* α-toxin was identified as a lecithinase by Macfarlane & Knight (1941), who suggested that this inhibition was due to a disorientation of the enzymes following disintegration of a lipoprotein complex. It seemed of interest to examine the action of the toxin on mitochondria, which, following the pioneer work of Bensley and his colleagues (Bensley & Hoerr, 1934) are known to be lipoprotein particles which swell in hypertonic solutions and possess various enzymic activities, including succinoxidase and adenosinetriphosphatase (ATP-ase) (Lazarow & Barron, 1943; Schneider, 1946).

The mitochondrial (large granule) fraction was prepared from rabbit liver and suspended in salt solution (0·14M-KCl, 0·012M-NaCl, 0·003M-$CaCl_2$). A typical suspension (30 ml.) from 60 g. liver contained per ml.: 2·2 mg. N; 0·18 mg. total P; 0·105 mg. ether-soluble phospholipin P, of which 50 % could be decomposed by *Cl. welchii* lecithinase; and 5·3 mg. ether-soluble lipoid. Enzymic activities were measured after short treatment with *Cl. welchii* lecithinase, using as controls the original suspension and a sample incubated with toxin and excess antitoxin.

Typical results indicating the complete inhibition of the succinic oxidase, succinic dehydrogenase and cytochrome oxidase (measured substantially according to Slater, 1949) of mitochondria by the toxin are shown in Table 1. With graded amounts of lecithinase the decrease in succinoxidase activity was paralleled by an increase in acid-soluble P indicative of the lecithinase action.

Table 1. *Succinoxidase activity of mitochondria*

(Five ml. mitochondria incubated with 10 enzyme units *Cl. welchii* lecithinase (approx. 20 LD_{50} mouse)±10 i.u. antitoxin at pH 7·2 and 37° for 10 min. O_2 uptake measured manometrically in air at 38° for 20 min. after equilibration.)

	μl. O_2 uptake/mg. N/hr.		
Treatment	Succinic oxidase	Succinic dehydrogenase	Cytochrome oxidase
None	294	125	110
Incubated with toxin-antitoxin	218	146	84
Incubated with toxin	Nil	7	4

The catalase and ATP-ase activities of the mitochondria were not affected by the action of the lecithinase. In earlier experiments it was found that the ATP-ase activity of twice-precipitated myosin from rabbit muscle was likewise unaffected by treatment with *Cl. welchii* lecithinase. This is in contrast to the finding of Kielley & Meyerhof (1950) that the unstable Mg-activated ATP-ase, free from myosin, is inactivated by *Cl. welchii* lecithinase.

The inhibitory action of *Cl. welchii* lecithinase on cytochrome oxidase appears sufficient in itself to account for the signs of toxaemia in clinical gas-gangrene.

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The Metabolism of Pregnane-3α, 20α-diol by Liver Preparations. By J. K. GRANT and G. F. MARRIAN. (*Department of Biochemistry, University of Edinburgh*)

A Reaction between Proteins and Glucose in Dried Human Blood Plasma. By C. H. LEA and R. S. HANNAN (*Low Temperature Research Station, University of Cambridge, and Department of Scientific and Industrial Research*) and R. I. N. GREAVES (*Department of Pathology, University of Cambridge*)

In previous work (Lea & Hannan, 1949, 1950*a*, *b*, *c*) it has been shown that a pronounced interaction takes place between casein and glucose when a freeze-dried mixture is held at moderate temperatures and humidities, the predominant reaction in the early stages being a combination of protein-free amino groups each with the reducing group of a sugar molecule.

A similar reaction, which has now been shown to occur between the proteins and glucose present in freeze-dried human blood plasma, is greatly increased when the plasma is admixed with extra glucose prior to drying, as is the normal practice in this country. The glucose content of the plasma used was 49 mg./100 ml. and of the glucose-enriched plasma 696 mg./100 ml., corresponding to 0·06 and 0·78 equivalents of the free amino-N present.

The rate and extent of the loss of free amino groups during storage depends on the water content of the material and on the temperature. The initial rate increased with increasing moisture content from zero when completely dry to about 10 % per day at 37° in glucose-plasma in equilibrium with an atmosphere of 55 % relative humidity. The temperature coefficient of the reaction is high. Small losses occurred even during drying.

As much as 5 % of glucose by weight can be firmly bound to the plasma proteins before there are any signs of change obvious to the user. Ultimately, discoloration and loss of solubility develop.

Nothing is yet known of the physiological properties of the compounds produced—virtually new proteins—which might thus be introduced into the blood stream in large quantity, and investigation is obviously desirable.

In view of these observations, it would seem advisable to reconsider the use of glucose in dried human blood plasma. The plasma should be dried to the lowest practicable moisture content, and stored at a low temperature with efficient exclusion of moisture. The same considerations can be expected to apply to other biological material containing proteins or amino-acids and reducing sugars which are preserved in the dry state.

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The Actions of Acetate and Other Buffers in the Assay of β-Glucuronidase. By LYNDA M. H. KERR, G. A. LEVVY and P. G. WALKER. (*Rowett Research Institute, Bucksburn, Aberdeenshire*)

Centrifuging water homogenates of normal, adult mouse liver at a high speed (20,000 g for 15 min.) caused separation of granules, probably mainly mitochondria, which contained about 50 % of the initial glucuronidase activity in the homogenate. Identical fractionation of the enzyme in mouse liver was obtained by centrifuging the homogenate at a moderate speed (2000 g for 15 min.) after addition of acetate buffer, pH 5–5·2. The enzyme in the granules was converted into a form soluble in the

acetate buffer by shaking with glass beads in a tissue disintegrator; by repeated freezing and thawing; and by addition of small amounts of Teepol XL. These measures did not inactivate the enzyme, but in higher concentration Teepol XL caused inhibition. The enzyme could also be extracted by washing the granules with citrate buffer, pH 5·2–5·4, but not by exhaustive washing with acetate buffer at the same pH. In the case of citrate, but not acetate buffer, small changes in pH and concentration modified the effect of washing on the enzyme in the granules. Other types of buffer ranged between citrate and acetate in their effects.

In livers from infant mice and livers regenerating after partial hepatectomy, the glucuronidase activity of the homogenate was higher than in the normal adult tissue (Levvy, Kerr & Campbell, 1948). Under these circumstances, a smaller fraction of the total activity was initially present in the granules.

The enzyme in the granules was gradually converted into a soluble form when they were maintained at 37° in acetate buffer at pH 5–5·2. This process was complete in 4 hr., after which inactivation became evident. Lowering the pH hastened the conversion into soluble enzyme. Since a similar change occurred in this fraction of the enzyme when crude homogenates were incubated in acetate buffer, it would appear that values obtained when acetate extracts are used for the assay of glucuronidase depend upon the duration of the incubation. As opposed to other workers in this field, we have used citrate buffer in preparing tissue extracts for glucuronidase assay (Kerr, Graham & Levvy, 1948). There was no change in the distribution of the enzyme when the homogenate was incubated in this buffer. A given enzyme solution in citrate buffer at pH 5·2 shows, however, only about 90% of the activity observed in acetate (see Oshima, 1936) at the same substrate concentration.

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A Glucuronide-decomposing Enzyme in the Rumen of the Sheep. By M. C. Karunairatnam and G. A. Levvy. (*Rowett Research Institute, Bucksburn, Aberdeenshire*)

An enzyme liberating free phenol and phenolphthalein from their respective glucuronides has been obtained in cell-free preparation from medium-sized micro-organisms in the rumen of the sheep. The pH optimum for the decomposition of phenolphthalein glucuronide (0·0125M) was 6·1 in citrate-phosphate buffer. The enzyme was rapidly inactivated below pH 5 and above pH 8. After bringing the preparation to pH 4·5 at 0° to coagulate inactive material, it was centrifuged and the enzyme in the supernatant was precipitated between 35 and 65% saturation with ammonium sulphate at pH 6·1. Inhibition by saccharic acid was slight, of the order of 20% with 10^{-2}M-saccharate. The activity of the enzyme was identical in pure citrate, phosphate or acetate buffer. It is considered that this enzyme may play some part in the breakdown in the rumen of grass polysaccharides containing uronic acid residues.

Albumin in Normal Urine. By J. R. Marrack and R. G. S. Johns. (*London Hospital Medical College, London, E.* 1)

Mörner (1895) found some 5 mg./100 ml. of protein in urine in which no coagulable protein could be detected by the usual tests; he considered that this was albumin. Wang & Wu (1937) found some 9 mg./100 ml. of protein separable from normal urine at a $CHCl_3$-water interface and Gunton & Burton (1947) some 3 mg./100 ml. by a spreading technique. The only way in which such small quantities of protein can be identified is by immunology. Nuttall & Dinkelspiel (1901) reported that a faint cloud formed when normal urine was added to the serum of rabbits immunized with human serum.

The best immunological method of making an approximate estimate of such small amounts is the optimum proportions method of Dean & Webb (1926). It is not so liable as other quantitative immunological methods to be vitiated by reactions with antibodies to impurities of the antigen with which the rabbits were immunized.

We have dialysed urine against water to remove the bulk of the salt and urea and then concentrated it, preferably by freeze-drying. The residue was dissolved in a small volume of saline containing phosphate buffer to bring the pH to about 7 and centri-

fuged. The concentration of albumin was then measured by the optimum proportions method at 38° using the serum of a rabbit immunized with human serum albumin.

Concentrations in four normal males ranged from 0·2 to 0·8 mg./100 ml. or 0·1–0·4 mg./hr. Moderate exercise seems to have little effect on the rate of excretion per hour.

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Hallachrome and Dopachrome. By J. D. Bu'Lock, J. Harley-Mason and H. S. Mason. (*Department of Organic Chemistry, University of Cambridge*)

Hallachrome is a red pigment from *Halla parthenopea* Costa, first studied by Mazza & Stolfi (1931), who announced its identity with the red oxidation product of tyrosine, the 5:6-quinone of 2:3-dihydroindole-2-carboxylic acid (Raper, 1927). A later refutation of this claim (Friedheim, 1935) attracted little attention, and the name hallachrome is still generally applied indiscriminately both to the *Halla* pigment and to Raper's 'red quinone', for which the name *dopachrome* is now proposed (A. H. Gowenlock, 1947, personal communication). Reinvestigation of hallachrome has revealed further clear distinctions from dopachrome in addition to the different solubilities in organic solvents (Friedheim, 1935). Hallachrome is not water-soluble at pH 5·6. Dopachrome has $\lambda_{max.} = 305, 475\ m\mu$. (water pH 5·4); the absorption spectrum of hallachrome is rather similar but has $\lambda_{max.} = 255,\ 313,\ 500\ m\mu$. (ethanol). In alkali, dopachrome reacts irreversibly; hallachrome gives, reversibly, a stable green colour with $\lambda_{max.} = 260,\ 365,\ 625–650\ m\mu$. Dopachrome is rapidly rearranged by acids to the colourless 5:6-dihydroxyindole whilst hallachrome gives, reversibly, a blue-violet compound with $\lambda_{max.} = 255,\ 310,\ 550\ m\mu$. The leuco-compound of hallachrome is yellow, with $\lambda_{max.} = 270,\ 375–400\ m\mu$.; leucodopachrome is colourless.

There are thus no grounds for applying the name hallachrome to anything other than the *Halla* pigment, work on the structure of which continues.

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The Effect of *cis*-Vaccenic Acid on Growth and Respiration of *B. subtilis*. By H. Laser. (*Molteno Institute, University of Cambridge*)

The essential nature of unsaturated fatty acids for the growth of certain bacteria is well established (see recent reviews by Pollock, 1949, and Kodicek, 1949). One of the most striking effects is the so-called 'double action' (Williams, Broquist & Snell, 1947), i.e. the very narrow margin between inhibiting and stimulating dose as well as the fact that the same dose, with time, may produce first an inhibition to be followed later by stimulation. In an attempt to throw some light on these phenomena, experiments have been carried out on the effect of *cis*-vaccenic acid [$CH_3.(CH_2)_5.CH{=}CH.(CH_2)_9.COOH$] on growth and respiration of *B. subtilis*. This acid has been shown to be a normal constituent of a number of animal organs and has previously been studied in relation to haemolysis (Laser, 1949; Morton & Todd, 1950).

The following are the main results. With the amounts of acid used (concentration in the medium = 1:12,500–1:100,000; ratio of acid/cells (dry weight) = 1:20–1:80) growth and respiration were inhibited for various periods of time proportional to the amount of added acid. The respiratory inhibition, however, decreased with time, leading at suitable concentrations to an increase in the oxidation rate above that of the controls. This applied to both resting and growing cells. Once respiratory inhibition has been overcome, growth may proceed at a rate even greater than that of the controls. Neither O_2 uptake nor growth of recovered cells is affected by a second addition of acid. The supernatant fluid of acid-treated, recovered cells causes an increase in the O_2 uptake of fresh cells.

It is believed that a purely physico-chemical theory in terms of surface phenomena cannot by itself account for all the observed facts. They can be more readily explained on the basis of (1) a penetration of acid into the cell; (2) an unspecific inhibition of the succinic oxidase system inside the cell (Slater, 1949); (3) the ability of the organism gradually to metabolize penetrated acid; and (4) the development in treated cells of a mechanism resembling that in acquired drug resistance.

Trans-vaccenic acid had only about half the activity of the *cis*-form.

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The Isolation of Lysine-free Hydroxylysine from a Gelatin Hydrolysate. By J. G. Heathcote. (*Department of Biochemistry, King's College, London, and the Research Association of British Flour-Millers, Cereals Research Station, St Albans*)

The isolation of a picrate possessing similar properties to those described for hydroxylysine picrate by Van Slyke, Hiller, Dillon & MacFadyen (1938) has been reported (Heathcote, 1948). The formation of mixed picrates and mixed hydrochlorides of lysine and hydroxylysine was adduced as evidence that, at the time, even the best samples of hydroxylysine contained lysine. The isolation of pure hydroxylysine has now been achieved by displacement chromatography.

A purified catholyte, prepared from a hydrolysate of gelatin, was freed from arginine by the addition of flavianic acid in stoichiometric proportions and then concentrated. From this concentrate the 'lysine' fraction was precipitated with ethanolic picric acid. After two recrystallizations the crude picrate yielded lysine picrate of the correct decomposition point (267°). The combined mother liquors from these recrystallizations yielded five crops (10 g.) of a more soluble picrate resembling that of hydroxylysine. From the first crop a mixed hydrochloride (melting range 150–250°) was prepared which was shown by paper chromatography to contain two basic amino-acids. Using phenol as solvent and atmospheres of ammonia and acetic acid respectively, two spots were obtained with ninhydrin, one of which gave consistent R_F values for lysine; the other, and slower moving, spot had a position to be expected for the more polar hydroxylysine. Dr A. H. Gordon at Copenhagen, using simultaneously this sample and one of hydroxylysine (containing lysine) obtained from Dr Van Slyke, confirmed this observation and identified the slower moving spot with the hydroxylysine of Van Slyke *et al.* (1938).

The pure base was finally separated from lysine by displacement chromatography on 'Zeo Karb–215' in the manner described by Partridge (1949), development being effected with 0·1 N-ammonia and followed throughout with paper chromatography. Hydroxylysine fractions from the column were combined and freed from ammonia by repeated evaporation to dryness. The base was then taken up in water, adjusted to pH 6 with dilute HCl, boiled with charcoal to remove traces of resin and concentrated. Addition of ethanol to the syrupy concentrate precipitated the monohydrochloride of the base as crystals which, after washing with ethanol and ether, had m.p. 233·5° (corr.). On repeating the experiment a fresh sample was obtained with the same melting point. The C, H and N analyses were theoretical for $C_6H_{14}O_3N_2.HCl$ and the conclusive nature of the separation was finally demonstrated by paper chromatography.

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The Biological Activity for the Rat of a Bound Form of Nicotinic Acid present in Bran. By D. K. CHAUDHURI and E. KODICEK. (*Dunn Nutritional Laboratory, University of Cambridge, and Medical Research Council*)

The presence in cereals of a bound form of nicotinic acid has been referred to by a number of workers (Kodicek, 1940; Waisman & Elvehjem, 1941; Snell & Wright, 1941). Krehl & Strong (1944) were able to prepare a concentrate of this bound form from wheat bran, which they called a 'precursor' of nicotinic acid. A more concentrated preparation of the 'precursor' from wheat bran has been obtained in this laboratory (Chaudhuri & Kodicek, 1950). The bound form of nicotinic acid is a poor source of the vitamin for lactobacilli, unless it is pretreated with weak NaOH or strong mineral acids. On the other hand, Krehl, Elvehjem & Strong (1944) found that a charcoal adsorbate of the 'precursor' was fully active for nicotinic acid-deficient dogs.

In the present experiments, rats were rendered deficient in nicotinic acid by giving a maize-sugar diet low in protein with which control animals respond well to graded amounts of nicotinic acid, as described by Harris & Kodicek (1950). The deficient rats were then given from the 17th day the 'precursor' preparation in daily doses of 2·5 or 5 mg. equivalent to 25 and 50 μg. of bound nicotinic acid. No growth response was observed. However, a full quantitative growth response was obtained when the 'precursor' preparation, prior to dosing, was hydrolysed by 0·1N-NaOH for 10 min. at room temperature and free nicotinic acid thus liberated.

Similar findings were obtained when wheat, rice and barley brans, unhydrolysed or hydrolysed with NaOH, were administered in amounts equivalent to 50 μg. of bound or free nicotinic acid. Apart from a small activity in wheat bran due to traces of tryptophan, the unhydrolysed brans were ineffective in curing the nicotinic acid-deficiency, while the hydrolysed samples were effective. It seems that the bound form of nicotinic acid present in brans is not available for the rat.

Experiments are in progress to confirm preliminary findings that the behaviour of maize is similar to that of the other materials tested. Evidence has been obtained that *Lactobacillus casei* can utilize fully the bound form of nicotinic acid present in maize and maize bran only after hydrolysis (Clegg, Kodicek & Mistry, 1950).

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Potassium Exchange of Brain and Retina. By H. A. KREBS, L. V. EGGLESTON and C. TERNER. (*Medical Research Council Unit for Research in Cell Metabolism, Department of Biochemistry, University of Sheffield*)

It was reported in previous papers (Krebs & Eggleston, 1949; Terner, Eggleston & Krebs, 1950) that slices of brain tissue and pieces of retina maintain their normal level of potassium if kept for 30–60 min. in a physiological saline containing glucose, L-glutamate and O_2. If one of these substrates is absent, K is lost by the tissue to the medium. On addition of the missing substrate the physiological K level may be restored.

It is generally accepted that differences in the ionic composition of animal tissues and their environment are the resultant of two forces: (1) passive diffusion, (2) active processes depending on energy supply. In other words, a system showing concentration differences represents a steady state and the problem arises of determining the rates of the two opposing processes. Ponder (1949) found for washed human red blood cells in saline that about 7 % of the cell K was exchanged per hour at 37°.

An attempt has been made to measure the rate of K exchange for brain and retina *in vitro* with the help of ^{42}K. Preliminary tests showed that even in the presence of glucose, glutamic acid and O_2 brain and retina lose K immediately after being placed in saline, but the initial level is restored within 30–40 min. and then remains approximately constant

for 20–30 min. The main experiments were carried out during this period of constant K concentration in tissue and medium. A solution of ^{42}KCl was added to the saline in a series of parallel cups after a preliminary incubation of 45 min. At intervals cups were removed from the water bath and tissue and medium were analysed for K and for relative radioactivity. From the loss of radioactivity of the solution the rate of K exchange was calculated. The following formula was used:

$$v = \frac{ab}{t(a+b)} \times 2{\cdot}3 \log \frac{bx_0}{x(a+b) - ax_0},$$

where v is the amount of K exchanging between tissue and medium per minute; a the quantity of K in the medium; b the quantity of K in the tissue; x and x_0 the relative radioactivity of the medium at time t and t_0.

Representative data are shown in Table 1. Consistent figures were obtained for v when calculated from different sets of measurements. Compared with red blood cells brain and retina exchange K very rapidly. In brain 3·5–4 % and in retina 8–10 % of the tissue K were exchanged per minute under the conditions of the experiments.

Table 1. *Potassium exchange of guinea pig brain-cortex and ox retina* in vitro

(Approximate amount of tissue: 100 mg. fresh weight.)

Tissue	Period of incubation (min.)	K in tissue (mM/kg.)	Radioactivity of K in medium (counts/min.)	Rate of K exchange (mM/min./slice)	Rate of K exchange in percentage of tissue K $\left(\frac{\text{K exchanged}}{\text{K in tissue}} \times 100\right)$
Brain	0	103	3275	—	—
	8	107	2687	$3{\cdot}30 \times 10^{-4}$	3·58
	12	94	2552	$3{\cdot}28 \times 10^{-4}$	3·33
	16	101	2381	$3{\cdot}28 \times 10^{-4}$	3·70
Brain	0	100	3470	—	—
	2	103	3275	$3{\cdot}40 \times 10^{-4}$	3·94
	4	96	3132	$3{\cdot}31 \times 10^{-4}$	4·11
	6	89	3050	$2{\cdot}96 \times 10^{-4}$	4·32
	8	91	2958	$2{\cdot}80 \times 10^{-4}$	3·67
Retina	0	68	4090	—	—
	2	73	3568	$7{\cdot}51 \times 10^{-4}$	10·7
	4	74	3162	$7{\cdot}75 \times 10^{-4}$	9·0
	8	94	2786	$6{\cdot}85 \times 10^{-4}$	8·2

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An Intestinal Inhibitor of Vitamin B_{12}. By G. E. Shaw. (*The Evans Biological Institute, Runcorn, Cheshire*)

By dialysing a 'salt-cake' made by adding NaCl to mucosa scraped from hog duodenum, which had been soaked in 0·2 % HCl, Bethel, Swendseid, Meyers, Neligh & Richards (1949) obtained a non-dialysable, water-soluble fraction which, although possessing but slight microbiological activity to *L. leichmanii*, would potentiate the therapeutic effect of orally administered vitamin B_{12}. Ternberg & Eakin (1949) found that normal gastric juice added to vitamin B_{12} would render this vitamin non-dialysable and non-available to micro-organisms such as *L. leichmanii*.

We have repeated Bethel's work using mucosa from the whole small intestine of swine. Saturation of Bethel's water-soluble fraction with $(NH_4)_2SO_4$ precipitates material inhibitory to vitamin B_{12} as shown by microbiological analysis using *L. leichmanii*. Similar material has been produced by extracting minced pig's small intestine with 0·2 % HCl and precipitating the extract with full saturation with $(NH_4)_2SO_4$. Work is proceeding on the fractionation of this material, and fractional precipitation with $(NH_4)_2SO_4$ will purify it to a considerable extent.

Most of the preparations possess some growth activity for *L. leichmanii*, this being apparently due to growth factors not inhibited by the inhibitor. Material obtained in a similar manner from human gastric mucosa had very little non-specific growth activity. The non-specific growth activity found in

the swine extract can be diluted out. These 'blank' dilutions still retain a useful amount of inhibitory power.

Microbiological analyses of the extract alone, boiled and unboiled, the extract plus a known amount of vitamin B_{12}, boiled and unboiled, are in accord with the supposition that the mucosa extract contains:

(*a*) Non-B_{12} growth factors.

(*b*) Inhibitor of microbiological activity of B_{12}.

(*c*) A complex of vitamin B_{12}-like material combined with the inhibitor which is dissociated by boiling.

Although the 'complex' is irreversibly split by heat, in no case have we been able to recover the full amount of added vitamin B_{12}.

The preparation also inhibits the growth activity of vitamin B_{12} for *Euglena gracilis*. The extract does not appear to promote the growth of *Euglena* in a non-specific manner.

No vitamin B_{12} inhibitor has been found in extracts of animal tissue other than those from the gastro-intestinal tract.

It is suggested that this preparation might prove a useful tool for the classification of growth factors of the B_{12} type.

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The Separation and Identification of Small Amounts of Mixed Amino Sugars. By E. F. Annison, A. T. James (Beit Memorial Research Fellow) and W. T. J. Morgan. (*Lister Institute of Preventive Medicine, London, S.W.* 1)

Examination, by means of paper chromatography, of the acid hydrolysis products of the mucoids which carry the human blood group characters indicates the presence of the two naturally-occurring amino sugars, glucosamine and chondrosamine, in each group substance. A method for obtaining further evidence for the presence of both hexosamines, based on the isolation of each amino sugar as a crystalline derivative, is now recorded.

The success which attended the use of 1:2:4-fluordinitrobenzene (FDNB) for the identification of the free amino groups of proteins and peptides suggested that the reactivity of the NH_2 groups in the amino sugars could likewise be utilized to form the corresponding 2:4-dinitrophenyl (DNP) derivatives.

The DNP derivatives were formed as follows. Amino sugar hydrochloride (20–25 mg.) dissolved at 0° in 1 ml. 0·4M-potassium borate buffer (pH 9·8) is immediately treated with a freshly prepared solution of FDNB (80 mg.) in 25 ml. of ethanol also at 0° C. The mixture is vigorously shaken, allowed to stand for 72 hr. at 0° C. and then taken to dryness under reduced pressure at this temperature. The DNP derivatives are recrystallized from one or more of the following solvent-systems: (*a*) acetone containing 15–25% water, (*b*) acetone-petroleum ether (b.p. 60–80° range), (*c*) methanol-benzene. DNP glucosamine, m.p. 201–203° (uncorr.); DNP chondrosamine, m.p. 182–184° (uncorr.). Yields, 80–90% of the hexosamine used.

Separation of the DNP amino sugars is accomplished by utilizing the difference in the configuration of the OH groups attached to C atoms 3 and 4 of the hexosamines. Glucosamine possesses *trans*-hydroxyl groups in these positions, whereas the corresponding groups in chondrosamine have a *cis*-configuration. Complex formation between the metaborate ion and *cis*-hydroxyl groups in carbohydrates is well known, and has been used by us in the belief that the 3:4 *cis*-diol of DNP chondrosamine would form a complex with the metaborate ion at pH 10·0 and that such a complex would show increased solubility in the aqueous phase of a partition chromatogram which employed as the solvent system 0·2M-KBO_2-KOH buffer (pH 9·7–10·0)/30% (v/v) amyl alcohol-chloroform, supported on a kieselguhr column. Glycine or carbonate-bicarbonate buffer of the same pH fails to bring about the separation of the DNP amino sugars. To avoid decomposition of the DNP derivatives at the alkaline pH employed the chromatograms are developed at 1–2°. Under these conditions no appreciable destruction of the DNP derivatives occurs, and their R_F values are sufficiently different to give rise to a good separation on the column described. The DNP chondrosamine issues from the column as a borate complex and must be recrystallized from aqueous solvents to dissociate the complex before DNP chondrosamine, m.p. 182°, is obtained.

By means of the procedure described glucosamine and chondrosamine have been confirmed as constituents of some animal and human blood group substances.

The Corneal Mucopolysaccharide. By A. M. Woodin. (*Ophthalmological Research Unit, Medical Research Council, Institute of Ophthalmology, Judd Street, London, W.C.* 1)

The mucopolysaccharide of cornea was described by Meyer & Chaffee (1940) as hyaluronosulphate, a sulphuric acid ester of hyaluronic acid which was hydrolysed by hyaluronidase preparations from pneumococcal filtrates and testis. It has been shown by Woodin (1950), that hyaluronidase preparations from several sources are without action as spreading factors in the cornea even under artificial conditions chosen to facilitate diffusion. The work of which this communication is a preliminary report was undertaken to prepare the corneal mucopolysaccharide free from protein and determine its activity as a substrate for hyaluronidase. The technique used to liberate the material containing ester sulphate from cornea is based on that used by Partridge (1948) to liberate the chondroitin sulphate from cartilage, since it was considered that such a method would give information on the state of combination of the mucopolysaccharide in the tissue.

The cornea contains 0·78% ester-SO_4 of which 60% can be removed by treatment with $CaCl_2$ (10% w/v) at pH 8. Dialysis of the $CaCl_2$ extract against water precipitates an insoluble mucoid (*NSM*) with N, 14·5%; ester-SO_4, 1·6%; hexosamine hydrochloride 4·2%. Remaining in solution is a soluble mucoid (*SM*) which can be recovered by precipitation with ethanol and has N, 12·3%; ester-SO_4, 3·5%; hexosamine hydrochloride, 10·1%.

Electrophoretic examination of *SM* at pH 7 revealed the presence of two components of which the first had hexosamine HCl/N (H/N) ratio of 3·5. A protein could be removed from *SM* by treatment with 92% phenol solution to leave a phenol-insoluble material (*PIM*), with N, 8%; hexosamine hydrochloride, 22·5%. *PIM* had two components in the electrophoresis apparatus at pH 8. The fast component constituted 85% of the total and had H/N = 3·5. Protein could not be removed from the *PIM* by the chloroform-amyl alcohol treatment, the interfacial precipitate having a composition identical with *PIM*.

Protein was removed by incubation with trypsin and the material recovered from solution had N, 2·8%; hexosamine HCl, 38%; SO_4, 17%; and was considered to be essentially protein-free.

Solutions of *PIM*, the fast component of *PIM*, and the mucopolysaccharide did not acquire reducing properties when treated with hyaluronidase under the conditions described by Rogers (1948).

The apparent intrinsic viscosity of the mucopolysaccharide is only 50% of that of *PIM*.

It is suggested that the soluble mucoid of cornea contains a protein-mucopolysaccharide complex of which the mucopolysaccharide is best described as a mucoitin sulphate.

The electrophoretic measurements were made by Dr R. A. Kekwick, to whom the author wishes to express his gratitude.

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Effect of Co-carboxylase on Formation of Acetate by Homogenates of Aneurin-deficient Pigeon Brain. By R. V. Coxon (Betty Brookes Fellow). (*Department of Biochemistry, University of Oxford*)

It has been shown (Coxon, Liébecq & Peters, 1949) that citrate and α-ketoglutarate are formed when pyruvate is metabolized by pigeon-brain homogenates and that the quantities formed are greater when fumarate or oxaloacetate is added with the pyruvate. In the absence of added dicarboxylic acid pyruvate tends to be preferentially converted to acetate in these homogenates. It was later found (Coxon & Peters, 1950) that the production of citrate and α-ketoglutarate was increased when the incubation was carried out in air as compared with nitrogen, and also, that in the case of homogenates prepared from aneurin-deficient brains, a further increase occurred when co-carboxylase was supplied *in vitro*. The present report concerns the effect of co-carboxylase on aneurin-deficient homogenates in the absence of added dicarboxylic acids. Although *in vitro* addition of co-carboxylase has no effect on homogenates from normal pigeons as regards the yield of acetate under such conditions, a substantial increase is induced by the vitamin in the case of homogenates from deficient birds. Since only very small quantities of acetate are formed anaerobically a lactate-acetate dismutation can be neglected in interpreting this result, which thus confirms the earlier suggestion that the first step in the oxidation of pyruvate is a decarboxylation, the resulting 2-C fragment being then either stabilized as acetate or

incorporated in a tricarboxylic acid depending upon whether or not there is a copious supply of oxaloacetate available. This accords with recent work on liver by Potter & Recknagel (1950), who found that pyruvate is incorporated either into citrate or acetoacetate depending on the availability of oxaloacetate in their preparation.

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The ATP-ase Activity of Isolated Myofibrils. By S. V. Perry. (*School of Biochemistry, University of Cambridge*)

Myofibrils prepared from rabbit skeletal muscle by the method of Schick & Hass (1949) are found to be unsatisfactory for enzymic investigations. Traces of trypsin remaining in the preparations produce considerable changes in the properties of the myofibrillar proteins, with the result that after several days' storage at 0° C., in some cases the turbid myofibril suspensions become almost clear. In such stored preparations the ATP-ase activity is maintained, but on addition of ATP to the 1·0M-KCl extract of the myofibril there is no decrease in viscosity such as is obtained when the extract is made immediately after the myofibrils are prepared. Myosin suspended in 0·05M-KCl, pH 7, and treated at 0° C. with 0·0045 mg./ml. crystalline trypsin, gives a clear solution within a few hours. The $CaCl_2$ activated ATP-ase activity shows no decrease, and in fact at first increases by 20–30 %, whereas the actomyosin-forming activity of the myosin falls off until it is almost negligible. Similar results with myosin have been obtained independently by Gergely (1950), and indicate that trypsin can modify the myosin molecule without destroying its enzymic activity. Increasing concentrations of KCl inhibit the ATP-ase activity of trypsin-treated myosin, in contrast to normal myosin or actomyosin which show maximum activity in 0·1M-KCl.

When myofibrils, which have been prepared by a modified method employing collagenase to break up the cell, are examined in the electron microscope they show considerable fine structure with no obvious differences from the normal picture. Low concentrations of ATP produce marked shortening yet considerable organization of the myofibrillar structure remains. Collagenase prepared myofibrils split ATP at rates roughly comparable to those obtained with similar concentrations of myosin, and show a small amount of myokinase activity. In borate buffer, pH 6·9, 0·002M-$MgCl_2$ gives maximum splitting of ATP at a rate only slightly less than that obtained when $CaCl_2$ is present at optimum concentration, i.e. 0·025M. In glycine, pH 8·6, 0·005M-$MgCl_2$ activates maximally to give a higher rate of splitting than can be obtained with $CaCl_2$ at this pH.

Unlike actomyosin, myofibrils show ATP-ase activity which is considerably enhanced by low concentrations of $MgCl_2$ in the presence of 0·1M-KCl. This activation frequently disappears on standing at 0° C. or incubation at 37° C., and after initially dissolving the myofibrils in 1·0M-KCl, $MgCl_2$ invariably decreases the ATP-ase activity measured in 0·1M-KCl. From observations on the effect of pH on this $MgCl_2$ activation and the fact that the collagenase preparations contained a small amount of lecithinase (which would presumably destroy the Mg-activated ATP-ase of Kielley & Meyerhof, 1948), it is concluded that the enzymic activity in the presence of $MgCl_2$ is a property of the actomyosin as it is arranged within the myofibril, and is not due to another distinct enzyme.

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PROCEEDINGS OF THE BIOCHEMICAL SOCIETY

The **288th Meeting** *of the Biochemical Society was held in the Department of Biochemistry, University New Buildings, Teviot Place, Edinburgh, on Friday,* 21 *July* 1950, *when the following papers were read:*

COMMUNICATIONS

A 'De-branching' Enzyme in Bean and Potato.

By P. N. Hobson, W. J. Whelan and S. Peat. (*Department of Chemistry, University College of North Wales, Bangor*)

In the course of an investigation of the Q-enzyme (Barker, Bourne, Wilkinson & Peat, 1950) of the broad bean we attempted to remove α-amylase impurity by absorbing it on starch grains from an aqueous ethanol solution of a Q-preparation (cf. Holmberg, 1933). Not only was α-amylase removed, but so also was Q-enzyme. The treated solution was without action on pure amylose, but it contained an enzyme (R-enzyme) having the novel properties of causing substantial increases in the iodine-staining powers (blue value) and limits of convertibility to maltose by β-amylase of both amylopectin and its β-amylase limit dextrin (β-dextrin). These reactions are not influenced by the addition of mineral phosphate. A summary of the properties of the new enzyme is given in Table 1.

Table 1. *Properties of R-enzyme*

	Action of R-enzyme	
Change in:	On β-dextrin	On amylopectin
Blue value	0·140→ 0·265	0·160→ 0·250
β-Amylolysis limit (%)	2·8 →61·6	48·4 →66·0
Reducing power (as % maltose)	0 →11·4	0 → 1·0
Viscosity (% fall)	85	51

The enzyme has been isolated as a stable powder from the broad bean and the potato. When it acted on whole potato starch or on amylose containing amylopectin the observed changes were in direct proportion to the amylopectin contents of the substrates, an indication that it is the branched, and not the linear, structures which are attacked. The increase in blue value which accompanies this 'de-branching' action would suggest that synthesis of 1:4 links is also taking place, with the formation of an amylose-type polysaccharide. It may be, however, that the action of R-enzyme is confined to the hydrolysis of 1:6 links and that the rise in blue value is due to a difference between the iodine-staining capacity of branched and unbranched chains. The products of R-enzyme action on β-dextrin or amylopectin are (*a*) not attacked by Q-enzyme, (*b*) of greater reducing power and lower viscosity than the untreated polysaccharides, and (*c*) have much lower blue values than amylose. These facts indicate that R-enzyme does not synthesize chain-forming (i.e. 1:4) links.

The simultaneous action of Q- and R-enzymes on amylose yields a product closely resembling an R-enzyme-treated amylopectin. R-enzyme has no action on unbranched chains of any length between that of amylose (approx. 200 units) and achroic dextrins (approx. 9 units), and is without influence on the synthesis of amylose by phosphorylase. These properties suggest that the de-branching action of R-enzyme is irreversible. In this case, the failure to achieve complete β-amylolysis of R-enzyme-treated β-dextrin and amylopectin is due either to the presence of two types of branch link, only one of which is hydrolysed by R-enzyme, or to a low affinity of β-amylase for the very short dextrin chains. The enzyme does not attack the 1:6 links in isomaltose or bacterial dextran.

An enzyme with a similar function has been isolated from muscle by Cori & Larner (1950).

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The Role of Z-Enzyme in β-Amylolysis. By GWEN J. THOMAS, W. J. WHELAN and S. PEAT.
(*Department of Chemistry, University College of North Wales, Bangor*)

In 1949, Peat, Whelan & Pirt reported that crystalline sweet potato and specially purified soya bean β-amylases effected a limiting conversion of amylose to maltose of only 70% instead of the almost complete conversion normally found with β-amylase preparations; a limit dextrin of high blue value (B.V.) remained. It was shown that the presence of a second enzyme (Z-factor) was necessary before complete β-amylolysis could occur.

The Z-factor has now been isolated as a stable solid by dissolving a standard β-amylase preparation from soya bean in 0·2M-acetate buffer, pH 3·0, heating for 1·5 hr. at 35°, adjusting the pH to 4·8 and freeze-drying. It is completely free from β-amylase and contains only a trace of α-amylase, the action of which is entirely inhibited if digestion is carried out at pH 4·8. The action of pure Z-enzyme on amylose and amylopectin is compared in Table 1 with that of α-amylase.

It is clear that although a mixture of α- and pure β-amylases acting on amylose simulates the combined action of pure β-amylase and Z-factor, the actions of the same enzyme mixtures on amylopectin are distinct enough to prove that Z-enzyme and α-amylase are not identical. Z-factor appears to eliminate some structure in the amylose molecule which forms a barrier to the action of pure β-amylase. This impeding structure is not a normal (1:6) branch link and is not contained in the 'outer' chains of amylopectin. Presumably, α-amylase circumvents the obstruction in amylose in a manner similar to that by which it by-passes the branch linkage in amylopectin, making both polyglucoses susceptible to further hydrolysis by β-amylase. When amylose is digested with Z-factor, the enzyme destroyed and pure β-amylase added the resulting conversion to maltose is 97·7%, proving that the action of Z-factor is not dependent upon the presence of β-amylase. When, in this experiment, α-amylase replaces Z-factor the limiting conversion to maltose is less complete, being 89·4%, a difference to be expected if α-amylase does not remove, but merely by-passes the obstruction which is eliminated by Z-enzyme; some parts of the starch chains would still be protected from the end-wise attack of β-amylase.

Table 1. *Action of enzymes on amylose and amylopectin*

(All digests incubated at 35·0° and pH 4·8. % conv. = % conversion to maltose; A.V. = absorption value (680 mμ.) of iodine-stained residual polysaccharide; β = crystalline sweet potato β-amylase; Z = purified Z-factor; α = freeze-dried salivary α-amylase; stock soya = stock soya bean β-amylase (containing Z-enzyme).)

	Amylose, B.V. 1·45			
	6 hr.		19 hr.	
Enzyme(s)	% conv.	A.V.	% conv.	A.V.
Z	0·0	1·40	1·3	1·39
α	1·8	1·37	—	—
β	67·3	0·357	67·5	0·340
β+Z	90·5	0·079	93·4	0·038
β+α	96·0	0·010	—	—
Stock soya	91·5	0·055	92·0	0·042

	Amylopectin, B.V. 0·158			
	2 hr.		7 hr.	
Enzyme(s)	% conv.	A.V.	% conv.	A.V.
Z	0·0	0·158	0·0	0·160
α	3·8	0·106	7·3	0·084
β	51·2	0·088	51·5	0·088
β+Z	52·3	0·088	51·5	0·088
β+α	58·3	0·032	64·9	0·023
Stock soya	50·9	0·095	52·5	0·073

Table 1 shows that Z-enzyme *per se* is without action on the 1:4-glycosidic links of either amylose or amylopectin, and that it cannot, therefore, be an amylase. It may be a phosphatase; the purified enzyme possesses powerful phosphatase activity with respect to glycerophosphate. On the other hand, the ester phosphate content of amylose is too low to admit of there being present even one phosphate group per amylose chain. Further experiments are in progress.

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The Determination of Reducing Sugar in Blood. By W. N. M. RAMSAY. (*Department of Biochemistry, University of Edinburgh*)

Reducing sugar can be determined in a $Cd(OH)_2$-$BaCO_3$ filtrate of whole blood by ferricyanide oxidation and photometric estimation of the resultant ferrocyanide after conversion to the pink ferrous-dipyridyl complex ion. As a routine procedure, duplicate analyses are made on a single specimen of 0·1 ml., but the sensitivity of the method permits scaling down so that a single analysis can be done on 0·02 ml. blood. The method is a little simpler and more rapid than the common volumetric procedures, and has the additional advantage that the final coloured product is a well defined stable substance.

The author wishes to thank Mr D. Purves and Miss D. C. Fairweather for their assistance.

The Configuration of the Anomeric Carbon Atoms in some Cardiac Glycosides. By W. KLYNE. (*Postgraduate Medical School, London, W.* 12)

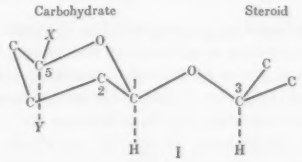
The method of molecular rotation differences (Barton, 1945) has been applied to the steroid glycosides. It can be used to indicate whether such compounds are α- or β-glycosides. All the cardiac glycosides to which the method can be applied probably have the same stereochemical type of union between steroid and carbohydrate.

Klyne (1950) has shown that the method of molecular rotation differences can be applied to structural problems concerning the cardiac aglycones and toad poisons. It has now been shown that in the steroid glycosides the rotation contribution of the carbohydrate component (ΔC) is almost independent of the nature of the steroid component:

Molecular rotation $= [M]_D = [\alpha]_D \times$ mol.wt./100,
$\Delta C = [M]_D$ of steroid glycoside $- [M]_D$ of free steroid.

The rotations of a series of synthetic steroid 3-glycopyranosides suggest the following general rule, namely, that the carbohydrate contribution ΔC is very approximately equal to $[M]_D$ of the corresponding α- or β-methylglycopyranoside.

A few compounds deviate considerably from this rule, probably because of unusual consitutional factors or solvent differences, e.g. the cholestanyl-glucosides of Linstead (1940). The rotations of these compounds were determined in pyridine, and some ΔC values calculated from them do not agree well with the $[M]_D$ values of the methylglucopyranosides in water and of their acetates in chloroform. However, the differences between the rotations of the anomeric pairs of cholestanylglucosides are all of the expected sign and order of magnitude. Apart from these exceptions the differences between ΔC and $[M]_D$ of the appropriate methylglucosides are between $+100$ and $-70°$ (mean difference 38° for a series of twenty-six compounds). These differences are much smaller than the differences in $[M]_D$ between anomeric α- and β-methylglycosides (350–500°).



I

The method of rotation differences may therefore be used to indicate the configuration at the anomeric carbon atom in naturally occurring steroid glycosides, if $[M]_D$ values for the corresponding methylglycosides are known. Table 1 shows the conclusions reached for a number of cardiac glycosides. It seems very probable from the conditions in which these com-

Table 1. *Probable configuration of the anomeric carbon atoms in some cardiac glycosides*

Carbohydrate	$[M]_D$ of methylglycopyranosides: α	β	Rotation contribution of carbohydrate in cardiac glycosides (ΔC)	Configuration suggested for anomeric C-atom
D-Cymarose	+370	(+ 40)*	−44, −22, −6, +22, +39	β
D-Diginose	+142	(−200)	−97, −156	β
D-Digitalose	+240	(−100)	−24, −44	β
D-Lyxose	+ 98	(−240)	−163	β
D-Sarmentose	+275	− 69	−119, −151	β
L-Rhamnose	−111	+170	−170, −178, −211	α
L-Thevetose†	(−260)	(+110)	−338	α†

* Values in parentheses are calculated from $[M]_D$ values of other derivatives.
† The natural acetyl L-thevetosides must have the same configuration.

pounds are hydrolysed that they are pyranosides and not furanosides. Comparison of the ΔC values of these glycosides with the rotations of the corresponding α- and β-methylglycopyranosides indicates that the D-glycosides are β and the L-glycosides α. It appears, therefore, that in all these compounds the stereochemistry of the union between carbohydrate and steroid is the same (I. For the β-D-lyxoside, $X = Y = H$; for the other β-D-compounds $X = CH_3$; $Y = H$; for α-L-compounds, $X = H$; $Y = CH_3$.)

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Studies on the Inhibition of Alkaline Phosphatase. By E. DICZFALUSY, B. ALDMAN, B. HÖGBERG and T. ROSENBERG (introduced by G. F. MARRIAN). (*Department of Women's Diseases, Karolinska Sjukhuset, Stockholm*)

The action of various inhibitors of the non-competitive type on the alkaline kidney phosphatase has been investigated. These inhibitors include the oestrogenic hormones, some of their derivatives as the phosphates, the glucoside, etc., and some synthetic compounds. Particularly strong inhibition has been found with oestradiol-3:17-diphosphate. The dependence of the inhibition on the pH has been demonstrated. The nature and mechanism of the inhibition and some biological relations were discussed.

Evidence for Transfructosidation in the Jerusalem Artichoke. By J. EDELMAN and J. S. D. BACON. (*Department of Biochemistry, University of Sheffield*)

When examined by paper partition chromatography the carbohydrates in extracts of the artichoke tuber appear as a series of ketose-containing spots; the lowest spot (1) has an R_F equal to that of sucrose, the highest (*n*) resembles inulin in having an R_F of zero (Bacon & Edelman, 1949). If aqueous extracts (pH 6·5) are incubated at 30° little change is noticed in the ketose content of the lower spots, but free fructose appears in measurable amounts (Edelman & Bacon, 1949).

When 1·5% sucrose was added and the extract incubated at 28° for 15 hr. the following changes were observed:

(1) Fructose was liberated, but in no greater amounts than in the same extract without added sucrose (fructose increased from 33 to 79 μg./20 μl.).

(2) The ketose content of spot 1 decreased markedly (from 299 to 252 μg./20 μl.).

(3) The ketose content of spots 3 to *n* decreased also (from 1219 to 1128 μg./20 μl.).

(4) The ketose content of spot 2 increased (from 143 to 236 μg./20 μl.).

No such changes were observed with boiled extracts; the reaction had a pH optimum of 6·0–6·5.

These results are most simply explained by assuming the transference of fructofuranoside residues from material of low R_F to sucrose, to give a trisaccharide (spot 2). This hypothesis was supported by a study of the action of dialysed carbohydrate-free enzyme preparations on mixtures of inulin and sucrose. Under these conditions spot 2, spot 3, and traces of higher spots appeared; the limiting velocity of the reaction was not reached in mixtures either with 5% sucrose (0·15M) or with 2% inulin (?0·004M). No effect of added inorganic phosphate could be demonstrated.

The extracts differ from levansucrase (Hestrin & Avineri-Shapiro, 1944) in showing little or no production of spot 2, or of higher spots, in the presence of sucrose alone, and glucose liberation does not appear to be a necessary part of the reaction, insignificant amounts appearing in tuber extracts, even on prolonged incubation with sucrose.

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The Influence of Sex on the Storage of Vitamin A. By V. H. Booth. (*Dunn Nutritional Laboratory, University of Cambridge, and Medical Research Council*)

When rats on a diet free of vitamin A were given vitamin A in any form (carrots, green leaves, halibut liver oil) more vitamin A was found in the livers of females than of males. This fact, though referred to in the literature, does not appear to have been studied. Males grew faster than females and lost their liver reserves of vitamin A faster. Hence the longer the interval between the beginning of dosing and the liver assay, the greater the sexual discrepancy. A difference was found, however, even when livers were assayed soon after a single dose. Since there had not been time for much growth, the storage difference could not be explained through the difference in growth rates. Moreover, in a group of rats of one sex given the same dose, although individual storage and individual growth both varied beyond the accuracy of measurement, there was no correlation between liver storage and growth rate: this applied to several groups of each sex.

Male rats are larger than females and therefore may have less 'surplus' for storage. However, the variation in storage by individual males of the same age and receiving the same dose showed no correlation with body weight. Nor was there a correlation among females. Further, young males from litters of twelve, though smaller than their female cousins from litters restricted at birth to two, still stored less than those females when given the same dose of halibut liver oil. Thus different body sizes of the sexes offers no explanation of the different capacities for accumulating vitamin A in livers.

The conclusion is reached that the difference between liver storage is not consequential to different growth rate or body size, but is a true sexual dimorphism. Although female rats lost their liver stores more slowly than males, females on a vitamin A-deficient diet barely outlived their brothers. An alternative storage site might explain this anomaly. The only significant reserves of the vitamin, elsewhere than in the liver, appear to be in the kidney (Johnson & Baumann, 1947) in which organ Moore & Sharman (1950), and also the author have found more vitamin in males than in females. The difference, however, is small and insufficient to counterbalance the superiority of female liver stores, but it is interesting through being in the opposite sense to differential liver storage.

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Vitamin A in the Kidneys of Male and Female Rats. By T. Moore and I. M. Sharman. (*Dunn Nutritional Laboratory, University of Cambridge, and Medical Research Council*)

Eden & Moore (1950) recently confirmed the observations of Johnson & Baumann (1947*a*,*b*) that when rats were given low doses of vitamin A, of up to about 30 i.u. daily, the concentration of vitamin A is higher in the kidney than in the liver. In preliminary experiments, moreover, they found that in male rats the kidneys contained more vitamin A than in females. This finding was in contrast with the general experience of Booth (1949), and others, that the livers of female rats tend to contain more vitamin A than those of males.

The results of further experiments on the distribution of vitamin A according to sex are given in Table 1. Young piebald rats were kept on a diet

Table 1. *Mean vitamin A in the kidneys and livers of rats receiving graded doses of vitamin A acetate for 24–36 days*

Vit. A, daily (i.u.)	Days of dosing	No. of rats of each sex	Kidneys				Livers			
			Males		Females		Males		Females	
			Total i.u.	i.u./g.	Total i.u.	i.u./g.	Total i.u.	i.u./g.	Total i.u.	i.u./g.
0	34	3	(0·9)	(0·8)	(0·8)	(0·7)	(7·1)	(1·8)	(1·7)	(0·4)*
10	36	3	3·4	2·0	3·3	2·3	8·4	0·4	2·5	0·4
20	35	3	16·4	8·8	10·0	8·1	6·9	0·6	30·3	5·2
40	26–33	7	41·0	24·1	6·4	4·9	88·5	9·4	160	24·4
80	24	4	29·2	18·6	5·1	4·3	396	45	720	115

* Maximum values, no blue colour observed with $SbCl_3$.

deficient in vitamin A, but adequate in vitamin E, for about 5 weeks, and were dosed daily with various levels of vitamin A acetate. It will be seen that after dosing with 10 i.u. of vitamin A daily the mean concentration of vitamin A was about the same in each sex, and exceeded the minute concentration in the liver. After doses of 20 i.u. the concentrations in the kidneys were somewhat higher and about the same in each sex, but the livers of the females contained much more vitamin A than those of the males. With 40 i.u. the concentration in the male kidneys was again raised without a corresponding rise in the female kidneys; the concentration in the males was thus about five times greater than in the females. At this level of dosing the concentration of vitamin A in the female liver was more than twice that in the male. With doses of 80 i.u. the concentrations in the kidneys were not further raised in either sex; the increased reserves in the liver still showed about the same ratio between the sexes. With one exception the levels of vitamin A in the pooled blood plasma of each group were greater in males than females, being 37 i.u./100 ml. at doses of 10 i.u., 56 at 20 i.u., 93 at 40 i.u. and 106 at 80 i.u. for males as against 31, 57, 79 and 73 i.u. for females.

Johnson & Baumann have observed that the concentration of vitamin A in the kidneys is usually high in rapidly growing animals. It remains to be decided, therefore, whether the high concentration in the male kidneys is directly due to sex, or indirectly to the well known superiority of the male over the female in growth rate. In continuing our experiments, trials are being made of the effect of sex hormones on the distribution of vitamin A.

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A Method for the Estimation of Small Quantities of Azide. By H. Lees. (*Department of Biological Chemistry, Aberdeen University*)

During the course of experiments on the oxidation of nitrate in soil it was noticed that azide, which was being used as a metabolic inhibitor, interfered with the estimation of nitrite by the Griess-Ilosva reagent. This reagent consists of two components: (1) sulphanilic acid which forms a diazo compound with the nitrite to be estimated, (2) α-naphthylamine which couples with this diazo compound to form a red dye. The intensity of the red colour formed by the overall reaction is therefore related to the initial concentration of nitrite and can be used to estimate it.

It was found that azide will react with the diazo compound and thus reduce the colour intensity produced on subsequent coupling with α-naphthylamine. The reaction is probably that referred to by Sidgwick (1937), in which an aryl azide results from the interaction of an aromatic diazonium compound and hydrazoic acid. The reaction can be made stoichiometric, and the reduction of final colour intensity caused by the addition of azide to a known amount of diazotized nitrite, prior to coupling with α-naphthylamine, can thus be used to estimate the amount of azide added. If a Hilger Biochem Absorptiometer fitted with a 4 cm. 25 ml. cell and an O.G. 1 filter is used, quantities of azide ranging from 0·1 to 0·3 μmol. may be conveniently estimated.

Trials have shown that azide added to fresh brain homogenates, to give final concentrations of 1×10^{-3} to 3×10^{-3} M azide, could be estimated in 0·1 ml. of the filtrate obtained after acid coagulation of the homogenate. The results given in Table 1 are typical.

Table 1. *Azide in* 0·1 *ml. filtrate* (μmol.)

Calculated from amount added to homogenate	Observed
0·33	0·34
0·26	0·27
0·20	0·20
0·13	0·13

The author hopes to use this estimation for investigating the precise action of azide on metabolic processes.

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The Response in Urinary 'Reducing Steroid' Excretion to Stimulation of the Adrenal Cortex by Adreno-corticotropic Hormone. By B. W. L. Brooksbank, Gwen M. Owen and F. T. G. Prunty. (*Department of Chemical Pathology, St Thomas's Hospital Medical School, London, S.E.* 1)

A preliminary investigation into the response to adreno-corticotropic hormone (ACTH) of 'reducing steroid' excretion has been carried out with the object of assessing the relative value of different techniques used in its assessment.

Urines have been extracted by hand at pH 1 immediately after completion of the 24 hr. collection. Extraction was completed in 2 hr. Sufficient urine was extracted to enable three methods of analysis to be employed on each sample.

(*a*) *Molybdate reduction* was performed on crude neutral extracts (Heard & Sobel, 1946; Heard, Sobel & Venning, 1946). The use of phosphomolybdic acid made with molybdic acid A.R. (Hopkin and Williams Ltd.) or from ammonium molybdate with sulphuric acid gave weak and unstable colours. The reagent was therefore prepared from molybdic anhydride (Harrington Bros. Ltd.) and gave $E_{1\,cm.}$ (using Ilford filter 608) 0·39 for 85·2 μg. deoxycorticosterone heated for 1 hr.

(*b*) *Formaldehyde estimation* was performed with periodate oxidation of fractions soluble in water following benzene : water partition (Daughaday, Jaffe & Williams, 1948). The residues were dissolved in 0·5 or 1 ml. purified ethanol before oxidation. Conditions for the maximum recovery of formaldehyde by distillation were carefully defined, this being in the range 88–95%. After correction for these losses deoxycorticosterone gave 100% theoretical colorimetric values. $E_{1\,cm.}$ using Ilford filter 606 was found to be 0·46 for 115 μg. deoxycorticosterone.

(*c*) *Copper reduction* was performed on the ketonic fractions, by a micro-adaptation of the method of Talbot, Saltzman, Wixom & Wolfe (1945). The method is sensitive over the range 6–90 μg. deoxycorticosterone. Interfering opalescence of the final solutions was reduced by the aid of ether extraction prior to measurement.

Table 1 shows some typical results obtained with several subjects.

Table 1. *Increase in urinary 'reducing steroid' after ACTH*

(i) = Basal level. (ii) = Maximum figure after ACTH.

			Deoxycorticosterone equivalent (mg./day)								
	ACTH		Molybdate (*a*)		Formaldehyde (*b*)		Copper (*c*)		Increase (mg./day)		
Subject	mg./day	Days	(i)	(ii)	(i)	(ii)	(i)	(ii)	(*a*)	(*b*)	(*c*)
F	50	2	2·1	6·5	0·45	3·63	0·50	1·89	4·4	3·18	1·39
	50	2	2·2	8·3	0·42	5·53	0·45	2·80	6·1	5·11	2·35
N	50	2	3·1	4·5	0·44	1·80	0·46	1·24	1·4	1·36	0·78
	50*	2	2·3	5·5	1·11	3·96	0·54	1·49	3·2	2·85	0·95
H	50	2	—	—	0·86	2·46	0·52	1·39	—	1·60	0·87
S	40	10	3·3	5·4	0·79	2·26	0·59	1·76	2·1	1·47	1·17
	50†	10	3·9	9·6	0·79	6·26	0·59	3·70	5·7	5·47	3·11
Pe	25	7	—	—	—	—	0·65	0·61	—	—	—
	25‡	7	—	—	—	—	0·40	0·69	—	—	0·29
Pa	25	7	—	—	—	—	0·70	0·78	—	—	0·08

* After 7-day interval. † After 10-day interval. ‡ After 21-day interval.

The results show comparable increases by methods (*a*) and (*b*) which are consistently greater than those by method (*c*), indicating the possible excretion of appreciable amounts of material with C-17-glycol side chains. Examination of some benzene fractions in method (*b*) shows that the differences between the increases obtained by methods (*a*) and (*b*) may be largely accounted for by the material in this phase. At the basal levels methods (*b*) and (*c*) give approximately the same figures, indicating the absence of non-ketonic substances in the water-soluble material.

17-Ketosteroid excretion was followed in parallel with these studies and was found to exhibit less consistent response to ACTH administration than did that of corticosteroid-like substances.

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Some Differences Between the Metabolism of 2-Naphthylamine and of 2-Acetamidonaphthalene in the Dog and Cat. By D. B. Clayson. (*Department of Experimental Pathology and Cancer Research, School of Medicine, University of Leeds*)

Wiley (1938) isolated 2-amino-1-naphthylsulphuric acid from the urine of dogs fed with 2-naphthylamine; Dobriner, Hofmann & Rhoads (1941) found 6-acetamido-2-naphthol to be a metabolite of 2-naphthylamine in the rat, rabbit and monkey.

2-Amino-1-naphthol cannot readily be diazotized, and therefore it was necessary to develop a method for its quantitative estimation. The method adopted depends on the light adsorption of a benzene solution of the purple pigment formed by aeration of an ammoniacal solution of the aminonaphthol. 50–500 μg. of the compound can be estimated to an accuracy of $\pm 5\%$ using the 1 cm. cells on the Hilger Spekker absorptiometer. 6-Acetamido-2-naphthol is easily diazotized after hydrolysis, and the total diazotizable amine was determined by coupling with *N*-sulphato-ethyl-*m*-toluidine.

Our experiments have shown that 30–70 % of the 2-naphthylamine administered to the dog is excreted as conjugates of 2-amino-1-naphthol. The percentage of the dose converted to this metabolite is smaller the larger the dose of 2-naphthylamine given. On the other hand, when 2-acetamidonaphthalene is administered to the dog the proportion of conjugates of 2-amino-1-naphthol falls to less than 5 %.

Similar results have been obtained with the cat; the amount of 2-amino-1-naphthol produced from the free amine has been shown to be greatly in excess of that produced from the acetamido-derivative.

The amount of diazotizable amine in the hydrolysed urine of the dog is small when 2-naphthylamine is administered, but is relatively large when the 2-acetamido compound is fed.

These results were discussed, and further evidence was presented to show that other acetamido compounds are not de-acetylated by the dog as the primary stage in their metabolism.

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Uptake of $^{32}PO_4$ by Flavin-adenine Dinucleotide of Rat Liver *in vitro*. By W. Good and G. Leaf. (*Biochemistry Department, University of Glasgow*)

Hummel & Lindberg (1949) reported that when rabbit liver mince was incubated with $^{32}PO_4$, flavin-adenine dinucleotide (FAD) took up ^{32}P with almost the same rapidity as adenosinetriphosphate (ATP). This has been confirmed using rat liver. In contrast to FAD and ATP, coenzyme I, adenylic acid and flavin mononucleotide do not appear to exchange their phosphorus under the same conditions. The implications of these findings were discussed.

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The Synthesis and Secretion of Amylase by Pigeon Pancreas Slices *in vitro*. By L. E. Hokin.* (*Medical Research Council Unit for Research in Cell Metabolism, Department of Biochemistry, University of Sheffield*)

The incorporation of a variety of labelled amino-acids into the proteins of tissue slices and homogenates (Melchior & Tarver, 1947; Anfinsen, Beloff, Hastings & Solomon, 1947; Winnick, Friedberg & Greenberg, 1947; Frantz, Zamecnik, Reese & Stephenson, 1948; Borsook, Deasy, Haagen-Smit, Keighley & Lowy, 1949) has been regarded as evidence of protein synthesis *in vitro*. However, a net increase of protein on incubation of an isolated tissue has not yet been reported. It was thought that a demonstrable net increase of a protein might be found upon incubation of a glandular tissue, capable of rapidly synthesizing enzymes which can be quantitatively determined.

Pigeon pancreas slices, which were depleted of their amylase content by supplying the birds with abundant food and injecting intramuscularly 0·1–0·15 mg. of carbamylcholine about 1 hr. before killing, were incubated at 40° under various

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conditions. The amylase activities of the medium and tissue were measured by the method of Smith & Roe (1949). By this procedure synthesis as well as secretion of amylase could be studied.

A progressive increase in 'total amylase activity' (sum of activities of tissue and medium) was observed when slices were incubated aerobically in saline media containing glucose. A greater increase occurred in serum containing added glucose. The increase was greatest (over 100% in some experiments after 2 hr. incubation) when a mixture of amino-acids was added (see Table 1). There was no increase in total amylase activity under anaerobic conditions, or in the presence of 2:4-dinitrophenol (10^{-4}M), cyanide (10^{-4}M) or iodoacetate (10^{-4}M). These observations indicate that the formation of amylase depended on a supply of energy and suggest that the increase in amylase activity represented a true synthesis of enzyme and not an activation of a closely related precursor.

In the presence of cholinergic drugs the fraction of amylase in the medium was significantly increased. This increase did not occur anaerobically or in the presence of 2:4-dinitrophenol (10^{-4}M) or cyanide (10^{-4}M). Thus pigeon pancreas slices appear to be capable of actively secreting amylase *in vitro*. Under the conditions of these experiments synthesis of amylase was not appreciably affected by secretion. *In vitro* secretion of an enzyme (pepsin) has also been reported by Edwards & Edwards (1949).

Table 1. *Synthesis and secretion of amylase by pigeon pancreas slices* in vitro

(2 hr. aerobic incubation. 0·2% glucose.)

Exp. no.	Conditions	Amylase activity (units of Smith & Roe mg. initial dry wt.) Medium	Tissue	Total
1	Unincubated slice	—	59	59
	Incubated in bicarbonate saline	27	40	67
	Incubated in inactivated sheep serum	26	67	93
	Incubated in bicarbonate saline with 0·4% tryptophan-supplemented acid casein hydrolysate	31	75	106
2	Unincubated slice	—	64	64
	Incubated in bicarbonate saline	28	78	106
	Incubated in bicarbonate saline with mixture of 21 amino-acids	32	115	147
	Incubated in bicarbonate saline with 0·4% tryptophan-supplemented acid casein hydrolysate	30	113	143

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Choline and Methionine in the Embryonated Hen Egg. By G. S. Boyd. (*Department of Biochemistry, University of Edinburgh*)

Seventy eggs incubated for periods between 0 and 21 days have been dissected after freezing in acetone-CO_2. Homogenized embryos and 'rests' ('rest' = yolk + white + allantoic and amniotic fluids + membranes) have been separately analysed for choline (method modified from Ramsay & Stewart, 1941, and Beattie, 1936) and methionine (McCarthy & Sullivan, 1941). In each case the substance is present practically entirely in chemical combination, choline as phospholipin, methionine in proteins.

In twenty unincubated eggs the total choline has been found to average 183 mg./egg (S.D. ± 22 mg.), and in eight unincubated eggs the total methionine has averaged 165 mg./egg (S.D. ± 11 mg.). Table 1 gives the results of the embryo analyses. Both compounds increase with incubation in the expected more or less smooth exponential manner, and methionine accumulates more rapidly than choline.

The analyses of the 'rests' show a progressive decrease, which in the case of methionine corresponds broadly to the increase in the embryo. In fact, up to the 18th day, the sum of the embryo and 'rest' methionine approximates closely to the total

methionine of unincubated eggs. At the end of incubation there is a fall, however, of about 25% in the total methionine of the egg, although the exponential increase in the embryo remains uninterrupted.

Table 1. *Analyses of embryos*

Incubation period (days)	Choline (mg./embryo)	Methionine (mg./embryo)
6	1	1
10	2	2·5
12	3	8
14	8	20
16	16	36
18	30	45
20	40	75

On the other hand, the total choline of the embryo and 'rest' is less than the choline of unincubated eggs as early as the 13th day, and by the 21st day the egg contains not more than 50% of the original 180 mg. choline. This decrease may be partly a coincidental reflection of the parallel change in lipoid phosphorus (Plimmer & Scott, 1908; Masai & Fukutomi, 1923; confirmed in this laboratory), but it appears probable also that choline is the only donor of methyl groups in the egg at least until the last 2 days of incubation. Such quantitatively important compounds as creatine and creatinine do not, however, account for more than 25% of the choline which disappears, even when the assumption is made that only one of the three choline methyl groups is available for transmethylation (cf. du Vigneaud, Chandler, Simmonds, Moyer & Cohn, 1946).

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The Behaviour of β-Glucuronidase and Nucleic Acids in Rat Liver during Growth. By G. T. Mills, Evelyn E. B. Smith, Beatrice Stary and I. Leslie. (*Biochemistry Department, University of Glasgow*)

A study has been made of the β-glucuronidase concentration in the livers of growing rats from before birth to maturity and in the livers of rats following subtotal hepatectomy.

It has been found that the liver β-glucuronidase concentrations are low in foetal rats, and that after birth there is a rise to a maximum around 20–40 days followed by a slight decline to an adult level above that for animals at birth. An examination of our data by the allometric method, which was first employed by Huxley (1924), indicates that the accumulation rate of β-glucuronidase is greater than that for liver tissue during the growth of the animal. However, the presence of other variables obscures the picture when liver weight is used as a base line and a much more precise index of reference than this is required.

Davidson & Leslie (1950) have demonstrated the value of the constancy of the deoxyribonucleic acid (DNA) content of the cell nucleus for a single species as an index of reference when studying growth phenomena. In the present work, by using the DNA content of the tissues as an indicator of cell number, along with the allometric method of analysis, the results indicate that the β-glucuronidase content per cell increases up to about 18 days post-partum and thereafter remains constant, and it is after this time that the growth rate of the liver is maximum. Our data reveal no relation between the rate of cell proliferation and the β-glucuronidase content per cell in the rat liver.

A study of rat liver regenerating after subtotal hepatectomy leads us to the same conclusion. In these experiments when the results are calculated on the basis of the DNA content of the tissue, a constant β-glucuronidase content per cell is found throughout the whole period of regeneration. During the 3 days following the operation when regeneration is most intense, the protein concentration of the liver is increasing rapidly while the β-glucuronidase concentration remains constant. It is only at about 6 days, when the protein concentration has returned to a constant level and regeneration is about 80% complete, that there is any significant rise in β-glucuronidase concentration.

The situation in the rat would therefore appear to be different from that in the mouse, where Levvy, Kerr & Campbell (1948) and Kerr, Campbell & Levvy (1949, 1950) have recorded a connexion between cell proliferation and the glucuronidase concentration of an organ. Further work is in progress to determine whether or not this is a true species difference.

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The β-Glucuronidase of *Escherichia coli*. By Evelyn E. B. Smith and G. T. Mills. (*Biochemistry Department, University of Glasgow*)

It was recently shown by Buehler, Katzman & Doisy (1949) that certain strains of *Escherichia coli* will produce β-glucuronidase, and that this production is stimulated by the presence of menthyl glucuronide in the medium.

With the strains of *Esch. coli* which have been examined in this laboratory, the presence of menthyl glucuronide would appear to be essential for the production of glucuronidase. On agar or in peptone medium, no glucuronidase is produced, while transference from the peptone to a similar medium containing 0·1% menthyl glucuronide stimulates glucuronidase production within 24 hr. For maximal production, growth in a medium containing 0·5% menthyl glucuronide is essential. Retransference of the adapted organism to an agar slope results in a 'lag phase' requiring several subcultures in 0·1% menthyl glucuronide to restimulate enzyme production. Growth is maintained in the adaptive state in a medium containing 0·1% menthyl glucuronide, but in media containing 0·5% menthyl glucuronide, the concentration of free menthol liberated enzymically becomes sufficient to inhibit growth completely.

It has been found that approximately 50% of the glucuronic acid present may be utilized by the organism during a period of 2 days' growth. Menthyl glucuronide, however, cannot be utilized as the sole source of carbon.

The rate of production of glucuronidase varies depending on the strain of *Esch. coli* used. In the strain investigated, enzyme production was maximal within 2–3 days (0·5% menthyl glucuronide), while in that kindly provided by Dr H. J. Buehler, St Louis School of Medicine, U.S.A., at least 10 days' growth in a similar medium was required to produce an equivalent enzyme preparation. Adaptation in the latter instance was also more prolonged.

In attempts to render the preparation cell-free by filtration, it was found that passage through a Seitz filter resulted in a complete loss of activity. The enzyme preparation was rendered cell free by centrifugation. Ultrasonic disintegration of the bacterial cell results in no increase in glucuronidase activity.

Dialysis resulted in a 95% loss of activity, which was not regained by combining the enzyme and dialysate, nor did the addition of glycine (0·01 M) reactivate the enzyme. In this respect the glucuronidase under study differs from that obtained by Buehler *et al.* (1949), which was apparently unaffected by dialysis.

The pH optimum for the hydrolysis of phenolphthalein glucuronide (0·00025 M) by this enzyme in phosphate-citrate buffer is 6·2, and the isoelectric point determined by the suramin method of Wills & Wormall (1950) is 5·6. Saccharate at a concentration of 10^{-3} M shows marked inhibition.

Further data were presented on the purification and properties of this enzyme.

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2:4-Dinitrophenol in the Study of Pathways of Pyruvate Metabolism in Lactating Mammary Tissue. By C. Terner. (*National Institute for Research in Dairying, University of Reading*)

In order to deplete lactating mammary tissue of preformed substrates, slices were shaken in three changes of ice-cold saline (Krebs & Eggleston, 1940) and then incubated at 37° for 1–2 hr. in fresh saline in equilibrium with 100% O_2. After a further washing in cold saline, 100–150 mg. portions of blotted slices were incubated in the Warburg apparatus. Depleted rat mammary tissue had a low endogenous Q_{O_2} (−2 to −3) compared with untreated tissue (Q_{O_2}, −4 to −6). The small increase of Q_{O_2} of depleted tissue on addition of glucose or pyruvate was prevented by 0·0025 to 0·01 M-malonate. When fumarate (0·002 M) was present, addition of pyruvate raised the Q_{O_2} to the same level as in untreated

portions of the same tissue (Q_{O_2}, -6 to -11; cf. Folley & French, 1949), and malonate did not prevent the increase of Q_{O_2} (cf. Krebs & Eggleston, 1940). Depleted slices and also homogenates oxidized succinic acid.

Addition of pyruvate in the presence of fumarate to depleted sheep mammary tissue usually increased the Q_{O_2}, but sometimes the increase was small or absent. However, subsequent addition of 2×10^{-4}M-2:4-dinitrophenol (DNP) increased the Q_{O_2} by 50–100%. A similar increase in Q_{O_2} in the presence of pyruvate and DNP was observed with rat, rabbit and goat mammary slices; there was, however, no corresponding increase in the rate of utilization of pyruvate, the ratio $\Delta Q_{O_2}:Q_{pyruvate}$ being less than 1 in the absence, and approaching 2 in the presence of DNP, indicating more complete oxidation of pyruvate in the presence of DNP. Representative results are shown in Table 1. Inhibitors of reactions of the Krebs cycle, malonate (0·002–0·01M), and fluoroacetate (0·005–0·01M) reduced both oxygen uptake and pyruvate utilization (see Bartlett & Barron, 1947; Liébecq & Peters, 1949).

Since DNP has been shown to inhibit synthetic reactions (Clifton, 1946) and to prevent phosphorylation (Loomis & Lipmann, 1948), these findings suggest that, apart from being oxidized via the Krebs cycle, pyruvate can be utilized in synthetic reactions in the mammary gland. In isotope experiments, pyruvate has been shown to be a source of fatty acid carbon *in vivo* (Anker, 1948) and in rat liver slices *in vitro* (Brady & Gurin, 1950). Fat synthesis by mammary tissue *in vitro* is indicated by the work of Folley & French (1950) in which utilization of acetate or glucose with high R.Q. is demonstrated. It is, therefore, of interest that an increase of Q_{O_2} on addition of DNP could also be observed in rabbit mammary tissue incubated with glucose plus acetate (Table 1), but not with glucose alone. Preliminary R.Q. measurements by the direct method of Warburg indicated a high R.Q. in the presence of pyruvate in sheep and rat mammary tissue which was reduced on addition of DNP. Acetoacetate did not accumulate in the presence or absence of DNP, and the accumulation of acetate is unlikely in view of its ready utilization (Folley & French, 1950).

The possibility of pyruvate being used for the synthesis of fatty acids by mammary tissue is being further investigated.

Table 1. *Effect of 2:4-dinitrophenol on metabolism of lactating mammary tissue*

(Rabbit mammary gland slices, incubated at 37° in phosphate saline without Ca (Krebs & Eggleston, 1940); gas, 100% O_2; Fumarate (0·002M) in all cups. Additions: pyruvate (0·005M), DNP (2×10^{-4}M), glucose and acetate (0·01M).)

Cup no.	Substrates added	Q_{O_2} during period (min.) 10–40	40–70	70–100	$Q_{pyruvate}$	$\frac{\Delta Q_{O_2}}{Q_{pyruvate}}$
1	None	−3·0	− 2·9	− 2·9	—	—
2	Pyruvate at 40 min.	−3·2	− 8·4	− 8·4	−6·1	0·85
3	Pyruvate at 40 min.	−2·9	− 6·4	− 6·4	−3·8	0·92
4	Pyruvate, DNP at 40 min.	−3·0	−15·1	−15·1	−7·8	1·55
5	Pyruvate, DNP at 40 min.	−3·2	−15·8	−15·8	−7·9	1·60
6	Glucose + acetate at 40 min. DNP at 70 min.	−3·1	−10·2	−16·9	—	—
7	Glucose + acetate at 40 min. DNP at 70 min.	−3·1	−12·0	−17·7	—	—

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The Metabolism of Acetate in Pigeon Breast Muscle. By Mabel Davison and Q. H. Gibson. (*Department of Physiology, University of Sheffield*)

Acetate is oxidized fairly readily by intact muscle cells (Lorber, Lifson, Wood & Barcroft, 1945; Medes, Floyd & Weinhouse, 1946; Villee & Hastings, 1949). The experiments reported here show that small amounts of steam-volatile acid disappear from muscle minced suspensions incubated with added acetate. The addition of boiled muscle or liver extract increases the steam-volatile acid disap-

pearance. This disappearance, if due to complete oxidation of acetate, would account for 6–15% of the total O_2 uptake. When either $^{14}CH_3COONa$ or $CH_3{}^{14}COONa$ was used, $^{14}CO_2$ was present in the respiratory CO_2. The effect of boiled tissue extract was to increase $^{14}CO_2$ formation as well as steam-volatile acid disappearance (Table 1). Succinate was isolated from experiments carried out with $^{14}CH_3COONa$ in the presence of 0·01 M-malonate. The succinate was found to be radioactive and to have a higher specific activity than that of the respiratory CO_2 (Table 2). This is regarded as evidence of formation of succinate from acetate in pigeon breast-muscle suspension. Boiled tissue extract increases the amount of labelled succinate formed.

Table 1. *Disappearance of steam-volatile acid and oxidation of acetate by minced pigeon breast-muscle suspensions*

(400 mg. wet weight muscle in 3 ml. phosphate saline, incubated 2 hr., 39°. Boiled tissue extracts (BTE) prepared from pigeon breast muscle, pigeon and rabbit liver. CH_3COONa oxidized calculated from $^{14}CO_2$ activity of respiratory CO_2.)

	Acetate oxidized (μmol.)	No. obs.	Steam-volatile acid disappearing (μmol.)	No. obs.
$CH_3{}^{14}COONa$				
No BTE	1·5±0·2	5	1·0±0·3	5
With BTE	3·3±0·4	10	3·8±0·5	8
$^{14}CH_3COONa$				
No BTE	1·0±0·2	7	1·1±0·2	7
With BTE	2·5±0·2	6	4·1±0·6	6

Table 2. *Formation of succinate from acetate by pigeon breast muscle*

(0·01 M-malonate, 30 μmol. fumarate/cup. Other conditions as Table 1. Acetate added gave 8900 counts/min. as $BaCO_3$ at infinite thickness. All counts expressed as counts/min. at infinite thickness, corrected for background, 11 counts/min.).

	Respiratory CO_2		Succinate	
	Counts/min.	Acetate (μmol.)	Counts/min.	Acetate (μmol.)
No BTE	20	0·1	114	0·6
BTE	29	0·1	251	1·3

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Observations on the Polysaccharides Composing the Cell Wall of Baker's Yeast. By D. J. Bell and D. H. Northcote. (*School of Biochemistry, University of Cambridge*)

The 'erythrocellulose' of Salkowski (1894) has been shown by Zechmeister & Toth (1934) and by Hassid, Joslyn & McCready (1941) to consist of two initially insoluble polysaccharides apparently both based on D-glucose. One of these is resistant to mild acid hydrolysis, the other is not. The latter has been termed 'pseudoglycogen' by McAnally & Maclean (1937). The Hungarian and American workers have both shown that the resistant 'glucan' is largely based on the 1:3-β-linked D-glucose radical. Bell & Northcote (1950), using chromatographic methods, have succeeded in accounting for nearly 100% of this substance and have shown that the polysaccharide is highly branched as follows: the average chain length is 9–10 radicals, the inter-radical links are 1:3, and the inter-chain link is 1:2. We have further examined the unhydrolysed pseudoglycogen which seems to be highly branched (mean chain length approx. 11 D-glucose radicals), it loses its iodine staining power under the action of salivary amylase and superficially resembles animal glycogens.

Since morphological relationships are assumed between red algae, yeasts and the algae components of lichens, this occurrence of the β-1:3 D-glucose linkage in yeast glucan is of interest. In red algae, 'Floridean starch' (Barry, Halsall, Hirst & Jones, 1949) and in lichenin from Iceland moss (Boissonas, 1947) these links are also found. In brown algae the polysaccharide laminarin possesses this link probably exclusively. The 1:2-β-D-glucan link has also been found in the 'hemicellulose' fraction from Iceland moss (Granichstädten & Percival, 1943). It should be noted that cell walls of brown algae appear to be composed of cellulosic material similar to terrestrial cellulose (Percival & Ross, 1949). A presumed reserve polysaccharide of brown algae, laminarin, is entirely composed of glucose radicals 1:3 linked according to Barry (1943). No evidence of the presence of amino sugars, alleged to occur in cell walls of fungi, has been obtained among yeasts or algae.

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PROCEEDINGS OF THE BIOCHEMICAL SOCIETY

The **289th Meeting** *of the Biochemical Society was held in Derby Hall, University of Liverpool, N. Mossley Hill Road, Liverpool* 18, *on Friday, September* 22*nd*, 1950, *and took the form of a Symposium on 'The Biochemistry of Fish'.*

COMMUNICATIONS

Some Comparative Aspects of the Biochemistry of Fish. By E. BALDWIN. (*Department of Biochemistry, University College, London, W.C.* 1)

The Proteins of Fish. By G. HAMOIR. (*Laboratoire de Biologie générale, University of Liège, Belgium*)

A review on the proteins of fish was published in 1948 by Geiger. The present report considered the more recent work and tried to give a general picture of the actual knowledge in the field.

An extensive study of the extraction of fish muscle has been carried out recently and the influence of different factors (grinding, ionic strength and pH of the extractant, nature of salt, cold storage) has been investigated (Dyer, French & Snow 1950; Snow, 1950; Subba Rao, 1948; Hamoir, unpublished).

The composition and some properties of the whole extracts have been examined. The presence of actomyosin, myosin, tropomyosin and myogen in fish muscle is now well established and their conditions of isolation are more accurately defined. Determinations of solubility, isoelectric points, water-holding capacity and H^+ combining capacity of some of these proteins have been carried out recently by Subba Rao (unpublished). Data relative to their ultracentrifugal and electrophoretic behaviour were presented (Hamoir, unpublished).

Recent publications in the field of blood proteins, collagens, proteins isolated from reproductive organs, and enzymes were considered.

More work is still necessary to define accurately the proteins of fish muscle and to improve their quantitative determination. A comparison of fish and mammalian muscle is already possible, however, which is of great interest from the points of view of comparative biochemistry and physiology.

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The Chemistry and Metabolism of the Nitrogenous Bases in Fish. By J. M. SHEWAN. (*Department of Scientific and Industrial Research, Torry Research Station, Aberdeen*)

The nitrogenous bases which account for 12–40 % of the total nitrogen in fish muscle, depending on the species, form a heterogeneous group of compounds and include the following: the volatile bases (ammonia, mono-, di- and tri-methylamines); trimethylammonium bases (trimethylamine oxide and betaines); guanidine derivatives (creatine, methylguanidine, etc.); glyoxaline derivatives (carnosine, anserine and histidine); urea; certain amino-acids and purine derivatives.

Present knowledge of the distribution of these compounds, particularly in the flesh of the edible European species, was summarized, it being noted that often little precise information exists concerning the presence of amounts of some of these bases, even in the commoner varieties. In the elasmobranchs about 65 % of the non-protein nitrogen can be accounted for as urea (50 %), trimethylamine oxide (6 %) and creatine (9 %). The remainder consists of ammonia and the lower amines, carnosine, histidine and possibly sarcosine, but at least 5–10 % is unaccounted for.

The physiological significance and technological importance of some of these bases, e.g. urea in the elasmobranchs, $(CH_3)_3NO$ in all species, and histamine in the *Scomber* group, has led to the accumula-

tion of many data on their distribution in relation to season, different body organs, environment, age and sex of the fish, and so on.

Particular reference was made to $(CH_3)_3NO$, for which data are more complete. It is believed to be an end-product of protein metabolism in fish and is said to play an important role, as does urea in the elasmobranchs, in the osmotic regulation of fishes.

The significance of trimethylamine oxide and its breakdown products—trimethylamine, dimethylamine and formaldehyde—in relation to the spoilage of iced wet fish, canned and other processed fish was also discussed.

The Chemistry and Metabolism of Fats in Fish. By J. A. LOVERN. (*Department of Scientific and Industrial Research, Torry Research Station, Aberdeen*)

The fats of fish contain a more complex mixture of fatty acids than do most land animal and plant fats. The acids normally range in chain length from 14 to 22 carbon atoms and include derivatives of varying degrees of unsaturation. Fish can readily digest and absorb high proportions of fat in the diet, even of fats solid at the body temperature of the fish. Absorption apparently takes place through the mucosal epithelium both of the intestine and its appendices and of the stomach. The nature of the dietary fat may have a marked influence on the fat of the fish, and general differences probably of such dietary origin are found between the fats of marine fish and those of fresh-water fish as a whole. Many fish specifically modify ingested fat, and mechanisms available include hydrogenation or dehydrogenation, shortening or lengthening of the carbon chain, and selective distribution in several depots. Mobilization of depot fat is non-selective as regards unsaturation but sometimes there seems to be some preferential mobilization of the acids of lower molecular weight.

Carotenoids in Fish. By T. W. GOODWIN. (*Department of Biochemistry, University of Liverpool*)

The carotenoid distribution in fish is characteristic in that carotenes, even β-carotene, are rarely found, whereas the xanthophylls encountered are restricted to three, viz. lutein, a carotenoid not as yet unequivocally identified but very similar to taraxanthin, and astaxanthin. Fish which accumulate lutein do not appear also to accumulate the taraxanthin-like pigment and vice versa, although astaxanthin is found associated with both. The major storage sites are the skin, muscle and ovaries (rarely, if ever, the testes). In the skin and muscle the xanthophylls are invariably esterified, in the ovaries they are free, whilst in the liver, mixtures of free and esterified pigments are often found. The pigments are actively mobilized into the developing ovaries from other regions of the body; in some species mobilization into the skin often takes place, especially in males, during the spawning period.

No animals can synthesize carotenoids *de novo*, but fish, together with many aquatic invertebrates, insects and birds, are able to produce highly oxygenated pigments by oxidizing alimentary carotenoids; they can also absorb carotenoids without altering them or, if the pigments have the required structural characteristics, they can convert them into vitamins A.

Recent work strongly suggests that a major function of carotenoids in fish is in photoresponses, for the characteristic pigments of the yellow and red chromatophores are very often lutein and astaxanthin respectively. It has also been claimed that they are important in reproduction, acting as fertilization hormones (*Befruchtungstoffe*).

It has long been doubtful whether the amount of β-carotene consumed by most fish is sufficient to account for all the vitamin A they accumulate and many possibilities, such as ascribing vitamin A activity to astaxanthin, have been considered. Recent observations that marine zooplankton contain relatively large amounts of vitamin A_1 indicate that most fish probably obtain much of their vitamin preformed.

Marine and fresh-water fish differ little in their carotenoid distribution and metabolism. This is in contrast to the case of the vitamins A; vitamin A_1 is the major component in marine fish, whilst vitamin A_2 predominates in fresh-water fish.

Bile Salts of Fish. By G. A. D. HASLEWOOD. (*Department of Biochemistry and Chemistry, Guy's Hospital Medical School, London, S.E.* 1)

The bile salts of about six species of elasmobranch (cartilagenous) fishes and of about forty species of teleostean (bony) fishes have so far been examined. From all the Elasmobranchii, scymnol has been isolated; the natural conjugate is a sulphuric acid ester. In addition it has been claimed that at least

two species of Elasmobranchii contain cholic acid; in one of these a C_{27} acid has been believed to exist.

In the Teleostei, which have been examined almost exclusively by Japanese workers, cholic acid has been stated to be present in all and chenodeoxycholic acid in many species. In four cases, a tetrahydroxy acid has been stated to occur. All these acids are conjugated with taurine. The author has found cholic acid as almost the only bile acid in the cod and plaice, but in a carp *Cyprinus carpio* var., the main biliary constituent appears to be a neutral substance, conjugated with sulphate. Japanese workers state that cholic acid occurs in this and an allied species.

The possible significance of these results was discussed in a consideration of the value of an investigation of the chemical nature of bile salts in different species.

Economic Aspects of the Fishing Industry. By C. L. CUTTING. (*Department of Scientific and Industrial Research, Torry Research Station, Aberdeen*)

Gross production and *per capita* consumption of fish vary greatly in different parts of the world. The principal fisheries of the world are pelagic fisheries producing fish such as herrings, pilchards and cod. Fish are less plentiful in tropical and subtropical waters than elsewhere, and it is noteworthy that the diet of people in adjoining land areas is frequently deficient in protein.

The important methods of preservation against bacterial spoilage for long term storage are the traditional processes of salting, alone (e.g. herring) or combined with drying (e.g. cod) or with heavy smoking (e.g. 'red' herring), and canning (e.g. salmon and tuna), which is a highly mechanized process. For quick distribution reliance is placed on the use of manufactured ice and light application of smoke. Freezing, for the long-term preservation of fish, necessitates continuous cold storage treatment and is still relatively undeveloped. About a third of the world's fish, comprising most of the pelagic catch, is reduced in specialized plants to meat and oil which often have low-grade industrial uses. The extent to which these industries are practised varies greatly from one country to another.

Although the world's fish catch could perhaps be increased by some 25–50%, there is probably an upper limit and already some intensively fished species in easily accessible waters show diminishing returns. Fish from the more prolific distant grounds cannot be landed fresh.

The main problems facing the fishing industry are the improvement of methods of preservation of all classes of fish for both developed and undeveloped countries, and the up-grading of the uses to which the catch, particularly that of pelagic fish, is put.

FORTHCOMING PAPERS

It is hoped to publish the following papers in the next issue of the *Biochemical Journal*:

Respiration and phosphorylation in preparations from mammalian brain. By E. M. Case and H. McIlwain

The inorganic phosphate and phosphocreatine of brain especially during metabolism *in vitro*. By H. McIlwain, L. Buchel and J. D. Cheshire

The reaction of oxidizing agents with wool. 3. The influence of the morphology on the rate of reaction. By P. Alexander, D. Gough and R. F. Hudson

The biosynthesis of choline methyl groups by the rat. By H. R. V. Arnstein

The inhibition of oxidative phosphorylation. By J. D. Judah and H. G. Williams-Ashman

The use of organic solvents at low temperature for the separation of enzymes. Application to aqueous rabbit muscle extract. By Brigitte A. Askonas

The preparation of permanent turbidimetric standards for use in the determination of small amounts of chloride. By J. Haslam and D. C. M. Squirrell

Estimation of protein in urine and C.S.F. with permanent turbidimetric standards of Perspex. By E. J. King

A simplified silver iodate method for the determination of chloride. By E. J. King and Dorothy S. Bain

Studies in the biochemistry of micro-organisms:

82. Ustic acid, a metabolic product of *Aspergillus ustus* (Bainier) Thom & Church. By H. Raistrick and C. E. Stickings

83. Fuscin, a metabolic product of *Oidiodendron fuscum* Robak. Part 2. Derivatives and degradation products. By J. H. Birkinshaw, A. Bracken, S. E. Michael and H. Raistrick

Studies in immunochemistry. 9. The oxidation of the human blood-group A substance with the periodate ion. By D. Aminoff and W. T. J. Morgan

Kinetic studies of the metabolism of foreign organic compounds. 1. The formation of benzoic acid from benzamide, toluene, benzyl alcohol and benzaldehyde and its conjugation with glycine and glucuronic acid in the rabbit. By H. G. Bray, W. V. Thorpe and K. White

The influence of 2:4-dinitrophenol on the oxidative breakdown of fatty acids. By P. Fantl, G. J. Lincoln and J. F. Nelson

A new volumetric method for the determination of histaminase activity in biological fluids. By R. Kapeller-Adler

The isolation of a toxic substance from agenized wheat flour. By P. N. Campbell, T. S. Work and E. Mellanby

The carbohydrates of the Jerusalem artichoke and other composites. By J. S. D. Bacon and J. Edelman

The separation of *N*-2:4-dinitrophenyl amino-acids on paper chromatograms. By S. Blackburn and A. G. Lowther

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